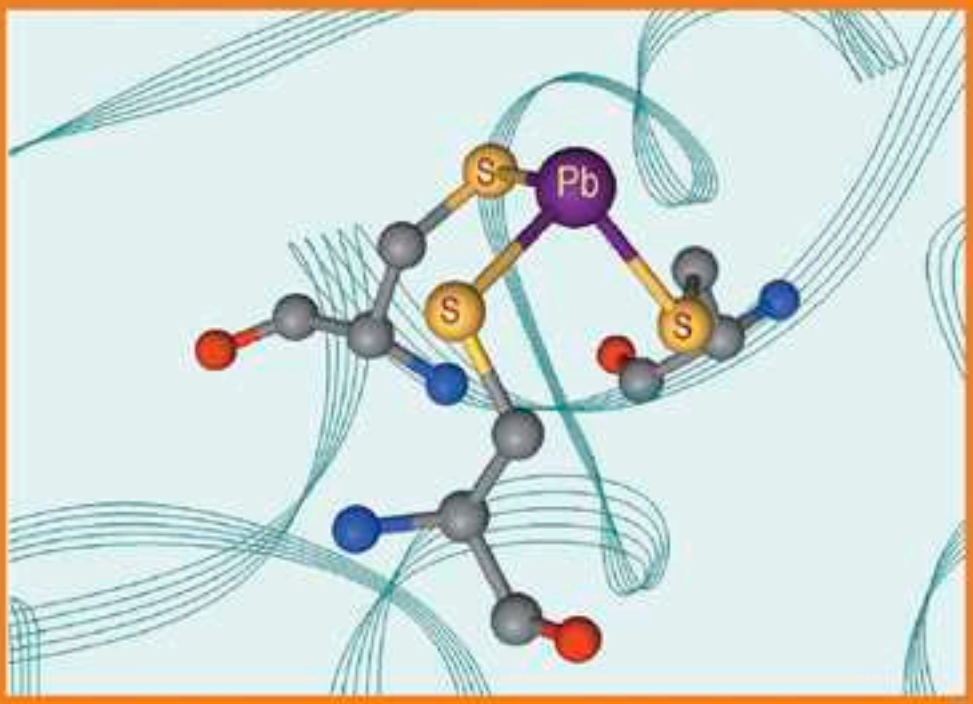
Lead

Chemistry, Analytical Aspects, Environmental Impact and Health Effects

Editors: José S. Casas and José Sordo



LEAD

Chemistry, Analytical Aspects, Environmental Impact and Health Effects

This page intentionally left blank

LEAD

Chemistry, Analytical Aspects, Environmental Impact and Health Effects

edited by

JOSÉ S. CASAS and JOSÉ SORDO

Departamento de Química Inorgánica
Facultad de Farmacia
Universidad de Santiago de Compostela
Santiago de Compostela, Galicia, Spain

ELSEVIER

Amsterdam – Boston – Heidelberg – London – New York – Oxford – Paris
San Diego – San Francisco – Singapore – Sydney – Tokyo

Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

First edition 2006

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN-13: 978-0-444-52945-9
ISBN-10: 0-444-52945-4

Printed and bound in The Netherlands

06 07 08 09 10 10 9 8 7 6 5 4 3 2 1

Preface

Lead has been with mankind almost from the beginning of civilization. Throughout this long journey to the present, this metal has been both angel and demon: in the words of John Emsley (The Elements of Murder, Oxford University Press, Oxford, 2005), "lead is useful, surprising, unpredictable, dangerous – and deadly".

The technical, economic and social importance of this metal is, at the present time, beyond all doubt. Nevertheless, over the last few decades surprisingly little attention has been pay in specialist literature to its behaviour (the properties and applications of its compounds, the environmental distribution of these derivatives, and their impacts on living creatures).

We hope that the present book will bring about a change in this situation. The book covers both "traditional" and recent advances in the field. It is not, strictly speaking, a comprehensive treatise dealing exhaustively with all the topics covered; however, it offers sufficient bibliographic guidance to allow exploration of most of these topics in depth. It includes coverage of historical aspects, lead mining and production, metal properties, common lead compounds, uses of lead and its derivatives, coordination chemistry, organometallic chemistry, environmental chemistry, toxicity mechanisms, and treatment strategies for lead poisoning. Finally, it describes analytical procedures for the determination of this metal in chemical, biological and environmental samples. This latter topic has given rise to an almost overwhelming literature in recent years, and the book offers a comprehensive review and summary of the procedures available. We hope that the book's integrated approach will be useful for students interested in a first contact with the chemistry of lead, but also for teachers and professionals working in technical contexts and in need of more specific information.

We wish to thank all the co-authors for their fine work and laudable patience throughout the process that finally crystallized in the present text. We also thank Elsevier for publishing it, and Joan Anuels from the Elsevier staff for her kind support. Finally, we express our gratitude to Prof. M.V. Castaño, Prof. M.S. García-Tasende and Prof. M.A. Sánchez-González for much helpful discussion and advice.

Santiago de Compostela, March 2006

The Editors

This page intentionally left blank

Contents

CHAPTER 5
Analytical procedures for the lead determination in biological and environmental samples 229

Pilar Bermejo and José A. Cocho de Juan

This page intentionally left blank

Lead - J.S. Casas and J. Sordo (Editors)

Chapter 1

An overview of the historical importance, occurrence, isolation, properties and applications of lead

José S. Casas and José Sordo

Departamento de Química Inorgánica, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

1. HISTORICAL IMPORTANCE

Nowadays lead and its derivatives have a very widespread use and world trade in this metal, either impure or refined, as well as in its minerals and compounds has been extensively developed. The large amount of the metal that is produced, the high economic value of its trade and the fact that its production and transformation employs a large number of people, all make lead an extremely important material. This situation is not new and there is evidence of the use of lead from very early times, well before the time of the Roman Empire [1]. Lead is one of the seven metals of antiquity, it was present in all the metal ages and has played a significant role in the progress of mankind.

The history of the element, the Latin *plumbum*, is largely related to the history of silver because, although silver occurs in the native state, the ores from which it was produced were lead ores from about 4000 B.C. The origin of the Latin term could be derived from a pre-Hellenistic language in the Aegean area or linked with an Iberian language, bearing in mind the abundance and ancient use of the metal in Spain

Ancient texts show considerable confusion between lead and other elements [1]; in fact, the term *plumbum* was originally used to describe any silvery white, low-melting and easily oxidized metal including lead, tin, zinc, and occasionally antimony and bismuth and its alloys.

The confusion with other elements was particularly significant between tin and lead. Plynium (cited in Ref. 1) refers to *plumbum nigrum* (lead) and *plumbum album* or *plumbum candidum* (tin); in his *Historia naturalis* he writes: *The next topic is the nature of lead, of which there are two kinds, black and white. White lead (tin) is the most valuable; the Greeks applied to it the name cassiteros, and there was a legendary story of their going to islands of the Atlantic Ocean to fetch it and importing it in plated vessels made of osiers and covered with stitched hides. It is now known that it is a product of Lusitania and*

Gallaecia found in the surface-strata of the ground which is sandy and of a black colour. It is only detected by its weight, and also tiny pebbles of it occasionally appear, especially in dry beds of torrents. Black lead does not occur in Gallaecia, although the neighbouring country of Biscaya has large quantities of black lead only; and white lead yields no silver, although it is obtained from black lead. Black lead cannot be soldered with black without a layer of white lead, nor can white be soldered to black without oil, nor can even white lead be soldered with white without some black lead.

The softness and lack of lustre made lead unattractive for jewellery or ornamental applications, but other interesting properties such as corrosion resistance, formability, malleability and low melting point brought about the large scale use of this metal, especially in Greek and Roman times.

A panoramic of the historical evolution of the use of lead, as well as some related issues such as production and poisoning, is briefly discussed below.

1.1. **Historical uses.**

Lead - either by itself or allied with other metals - was widely used [1] in water piping, for architectural and engineering applications, for statues, figures, weights and coins. Furthermore, compounds of lead were used in glass, glazes and enamels, pigments and paints, cosmetics and medical applications.

Lead pipes have been used since ancient times. Very old pieces have been recovered from Mesopotamia, Cyprus, Persia, Egypt, Greece, Rome and the provinces of the Roman Empire. The Romans, who could have learnt about lead plumbing technology from the Greeks, demanded large quantities of water. The pieces recovered from aqueducts prove that the mainstay of their water-distribution system was lead piping. In addition to piping, the water supply system included the use of tanks, baths or vats and several ancient examples of these have been found.

The positive qualities that make lead useful for the plumbing industry, such as malleability, durability and resistance to corrosion, are sharply counterbalanced by the potential health hazards, which will be discussed later.

Lead was also used in many instances where iron wire or wooden hoops are currently used; for example, amphorae used to store water or wine were reinforced with bands of lead. Other examples of the use of lead in ancient architectural practices include joining masonry, cesspool coverings, roofing, damp-proofing of foundations, parapet walls and for openings in buildings.

Lead sheets or tablets were used for inscriptions and lead seals used to be attached to messages and merchandise. Lead statues, statuettes and figures, weights and coins have been found from many ancient cultures. Lead vessels and kitchenware - already used in ancient Persia, Egypt and Greece - were widely used during the Roman Empire.

Lead has been used since ancient times for purposes connected with the burial of the dead. A large number of coffins and urns from the Roman period

have been found, many of them in England, which may be due to the abundance and low value of lead in this area during this period.

In most of the aforementioned applications lead was allied with other metals. Metallic artifacts containing lead dating from the Bronze Age were found in Babylonia, Egypt, Greece and other areas of Europe and China. Examples of leaded bronzes include Egyptian statues (up to 25% lead), ancient Chinese bronzes (up to 50% or more and for the most part a ternary copper-lead-tin alloy) and highly leaded bronzes from the late Bronze Age or Iron Age from Iberia and the British Isles.

In Greece, copper-lead-tin alloys were quite common during the Archaic, Classical and Hellenistic periods. The deliberate addition of lead to statuary and coinage bronze took place during the Archaic period. The lead concentrations varied, with the Hellenistic average lead content being over 13%, although in specific pieces up to 30% lead has been found.

The Greek use of lead in bronze was adopted and even extended by the Romans to produce, for example, statues and coins and also for architectural and engineering purposes.

In addition to bronzes, pewter was also widely used. The variable composition of this tin-lead alloy was influenced, among other factors, by the relative prices and the availability of the two metals and also by the usual practice of remelting old pieces.

Another type of tin-lead alloy is solder, which is used for sealing and joining metals. The different uses of the alloy define the composition, which ranges from 38 to 98% lead [1].

A number of representative examples to illustrate the wide compositional variety of ancient bronzes, pewter and solders are shown in Table 1 (adapted from Ref. 1).

Besides these applications of the element in its metallic form, several lead compounds, as mentioned above, have been used in glass, glazes, enamels, pigments and paints since Antiquity. Lead compounds were also used in cosmetics and medicines.

The compounds used in all of these applications could either be mineral or manufactured because even though the manufacturing of lead compounds on a commercial scale began in Greco-Roman times, lead minerals were in fact mined, transported and used a long time before.

Galena, lead(II) sulphide, is essential for the production of lead and silver, but it was also used as eye paint in Egypt and in the manufacture of lead glazes and glass as well as for medicinal purposes in Greece and Rome.

Variable proportions of red (tetragonal form, litharge) and yellow (orthorombic form, massicot) lead(II) oxide were identified as components of glass and glazes from Greco-Roman times and from ancient Egypt and Assyria.

Table 1
Representative composition of ancient "bronzes", pewter and solder

	Alloy	Description	Date of Issue/ /Age	Composition (wt %) Cu	Pb	Sn	Ag	Ni
China	"Bronze"	Coin	770-249 B.C.	38.3	55.4	1.7		1.0
China			722-481 B.C.	70.4	19.3	9.9		0.35
China			340-325 B.C.		62.0	1.8		0.25
Egypt			285-246 B.C.	85.61	0.53	12.37		
Macedonia			227-239 B.C.	88.6	4.45	6.48	0.03	
Spain			14-37 A.D.	92.1	0.15	7.2	0.10	
Spain			14-37 A.D.	77.0	15.2	7.3	0.10	
Rome			37-41 A.D.	99.24	0.46	0.10		
Egypt			117-138 A.D.	63.66	30.00	6.09		
Rome			308-310 A.D.	82.0	9.8	6.1	1.6	
Rome			Republic	72	28			
Rome			Republic	65	30	5		
Rome			Republic	92	1	6		
China			1092 A.D.	57.0	28.5			
England	Pewter	Cup	Roman		54.80	45.38		
England		Coffin	Roman		55.31	44.97		
England		Coffin	Roman		58.48	41.84		
England		Seal	Roman		72.90	27.10		
England		Dish	Roman		57.0	43.0		
England		Button	Roman		0.82	99.43		
England		Cup	Roman		2.73	97.70		
England		Strip	Roman		4.50	94.50		
England		Ring	Roman		33.53	66.79		
England		Tableware	Roman		37.8	62.2		
England		Tableware	Roman		24.	76.		
England		Tableware	Roman		20.5	79.5		
England	Solder		Early Iron Age		70.0	16.5		
England			Roman		73.6	25.3		
England			Roman		61.8	38.0		

(Adapted from Ref. 1)

Lead(II) oxide was also used to manufacture glass in ancient China (highly leaded glass contains more than 30% Pb), where was also widely used as make-up.

Another lead oxide, Pb_3O_4 (minium or red lead), was used as a pigment for paintings in ancient times, despite the fact that it is unstable in air and changes to a brown colour when exposed to light. However, the most important lead pigment was white lead or cerusse, a basic lead(II) carbonate for which an ideal composition would be $2PbCO_3.Pb(OH)_2$, although in reality it was a mixture of $PbCO_3$, $Pb(OH)_2$ and PbO in variable proportions. Although lead(II)

carbonate is a fairly common mineral known as cerussite, the basic lead carbonate used as a pigment was manufactured by the ancient Greeks, Romans and Chinese, who used it for painting pictures, in cosmetics and occassionally to decorate walls.

Most of these applications of lead compounds have recently been banned or reduced but others are still carried out today, as we shall describe later; however, a new and important application was developed and phased out in the 20th century, namely the use of the organolead compound tetraethyllead (TEL) as an antiknocking agent in gasoline.

The rise and fall of this compound, from its synthesis and characterization in European universities, to the further study and exploitation as an antiknocking agent in the USA, was recently revised by Seiferth [2]. The great economic and environmental importance of this compound (279000 metric tons of lead consumption in 1970 in the manufacture of organolead antiknocking agents in the USA [2]) deserves a specific space in this historical description, underlining several points of Seiferth's review.

Although TEL was isolated and characterized as a pure compound by G. B. Buckton in 1859 by using the reaction of diethylzinc with an excess of lead dichloride, other authors had previously synthesized the impure compound by reacting ethyl iodide with a sodium/lead alloy. After the publication of Buckton's work, other authors improved both types of syntheses and further characterized TEL, such as A. Cahours, who described the synthesis of tetramethyllead in 1861. After the discovery of the Grignard reagents in 1900, both compounds were prepared by reacting the corresponding methyl- or ethyl-Grignard reagent with lead(II) chloride in ether.

All of these academic efforts to synthesize and characterize TEL were similar to those made for other organometallic compounds. But, as Seiferth points out: *It was the phenomenon of "knock" that occurred during the operation of the gasoline engine of the automobiles in the early 20th century that raised TEL out of the "noise" of the many known organometallic compounds of the day to its stellar prominence as the most commercially important member of this class.*

The knock is the detonation of a small part of the less volatile, unburned fuel/air mixture that remains in the cylinder of an internal combustion engine after the completion of the cycle. This phenomenon prevented the development of more efficient and more powerful high-compression automobile engines and so the search for an effective and practical antiknocking agent became an industrial challenge at the beginning of the 20th century, a time when the development of better fuels and better engines was constantly changing.

After a series of laborious processes, during which a lot of compounds were tested, the strong antiknocking effect of TEL was discovered on December 9th 1921, at the General Motors laboratories. After additional studies, the compound was incorporated into gasoline and so the first leaded gasoline was

sold in the USA on February 1st 1923 in Dayton, OH. Afterwards the production of TEL increased drastically and as a result the known synthetic procedures were re-investigated and improved, as described by Seiferth [2]. The reaction of ethyl chloride with a sodium/lead alloy was especially studied, improved and widely used to manufacture TEL.

Public concern about the toxicity and environmental effects produced by this additive, which will be described later, led to a significant and continuous reduction of industrial production after 1975 [3].

In the USA the use of TEL as an antiknocking agent in gasoline for on-road use has been prohibited, following a continuous decrease of permissible lead levels (from 1.7 to 0.5 g/gal from 1975 to 1979 and to 0.1 g/gal by 1986). A similar strict regulation has been adopted in the European Union and in other countries such as Canada, Mexico, Switzerland, Brazil, Argentina, Australia, New Zealand, Russia, Japan, China, Taiwan, Korea, Singapur and India [2]. However, the sale of leaded gasoline for this use is still permitted in several countries in Africa, the Middle East, Asia and Latin-America and in some countries an organolead concentrate can be purchased to add to gasoline for specific uses.

The medicinal applications of lead and its compounds in ancient times were significant. Hippocrates, Galen, Dioscorides and Paracelsus described several lead preparations that were useful in curing a variety of illnesses. This practice was general even though the poisonous effects of lead had been known since ancient times. These applications were, however, progresively phased out as knowledge of the toxicological effects increased and were more widely understood.

1.2. **Historical production and resources**

The difficulty in providing a rigorous description of lead resources and production levels in ancient times was highlighted by Nriagu [1]. However, from numismatic literature, archeological evidence and from data on silver (a very strongly related metal from the point of view of production) and other precious metals, Nriagu estimated the evolution of the cumulative production in different geographical areas during ancient times [1, 4]. The findings are represented in Figure 1. The evolution of these production levels in some areas warrants further comment.

In the Aegean Islands, Crete and Greece production reached a maximum during the Bronze Age, although in the Iron Age it still remained significant. In the Bronze Age, although lead was mined on several islands, the main production came from the Laurion mines in southeastern Attica, Greece, probably the principal source of lead and silver in the eastern Mediterranean.

The combined production of the Iberian Peninsula in the period from 3900 B.C. to 1000 A.D. is the largest of all the geographical areas listed in Figure 1. Although lead, and in general the mineral resources of Portugal and Spain, was

coveted and exploited by the Phoenicians and Carthaginians, the mining activity was also very significant under the Roman Empire. The southern and southeastern regions in general and those areas close to Cartagena and to the Sierra Morena mountains in Spain were particularly productive.

Other European areas also produced significant amounts of lead, albeit at a lower level than the two regions discussed above. The exploitation of the lead-silver resources of the British Isles during the Copper and Bronze Ages is not well documented and it was during the Iron Age that lead metallurgy seems to

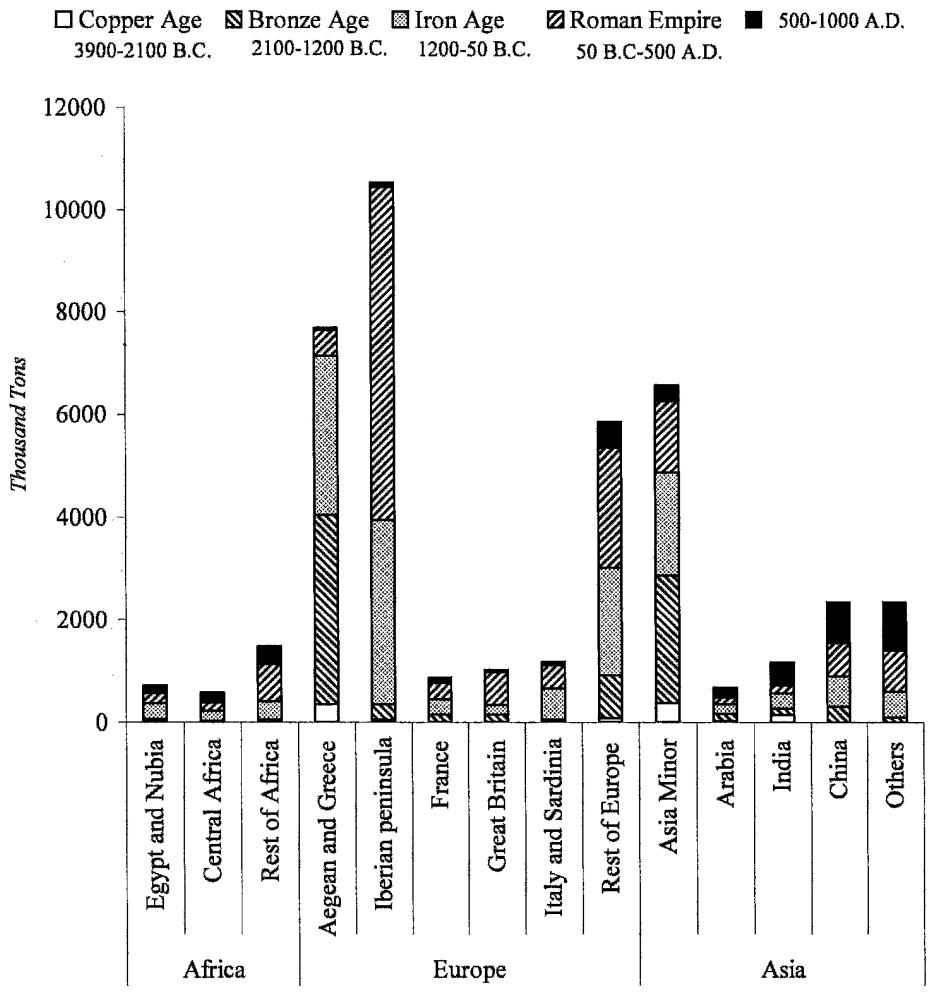


Figure 1: Lead production in the Ancient World (Data from Ref. 1).

have become well-established. Lead production increased after the Roman conquest; the ores were abundant and rich and were also near the surface, a situation that led to rapid and cheap extraction. This fact could be responsible for the decay of the Spanish lead mining industry after the opening of the British mines.

The exploitation of silver-lead ores in Sardinia, previously mined by the Phoenicians, was rare between the Carthaginian and Roman times but developed once again under the Roman Empire; around 400 A.D. the lead from Sardinia constituted a significant proportion of Roman lead production. These types of ores had also been exploited in the Italian Peninsula since ancient times. Although the available information from the Bronze Age is limited, the Etruscan exploitation of the mineral resources in Tuscany during the Iron Age is well documented. Silver production in Italy fell during the Roman Empire, partly due to the comparatively low profitability of the ores and also to several Senate regulations limiting extraction.

The exploitation of lead-silver ores in France was important before the Roman conquest but during the Roman Empire only limited quantities of these elements were obtained; archeological evidence suggests that the production of these two metals in Germany and central Europe during the Roman Empire was not very extensive. In the Balkans the mining industry was significant in both pre-Roman and Roman times. Examples of this exploitation include Macedonia during the Hellenistic times and, in Roman times, all of the area that encompasses modern Serbia, Bosnia, Rumania and Bulgaria. Production decreased and was practically halted at around 400 A.D.

Asia Minor and China were other active areas in Antiquity. From 3500 B.C. lead and silver were produced in Asia Minor and exported to Mesopotamia, where they were widely employed. In China, lead ores were widely distributed, particularly in the southwestern provinces. The element was used in leaded bronzes from about 1750 B.C., and was also a component of other alloys. Lead compounds were used in applications ranging from cosmetics to paints and health care.

The total amount of world lead production during the Copper, Bronze and Iron Ages reached levels of 1050, 8490 and 14310 thousand tons, respectively [1], showing a marked increase with time. During the Roman Empire the production reached 14960 thousand tons and then declined, with only 4250 thousand tons produced in the period 500-1000 A.D.

Settle and Patterson [5] described the evolution of world lead production (in tons/year) throughout History, estimated on the basis of data regarding silver production. As shown in Figure 2, the introduction of silver coinage created a demand for silver, which resulted in an increase in global lead production. This reached a value of about 10000 tons/year when Athens flourished. Production increased in Roman times, giving a maximum world value of about 80000 tons/year at the beginning of the first century A. D., a time that coincided with

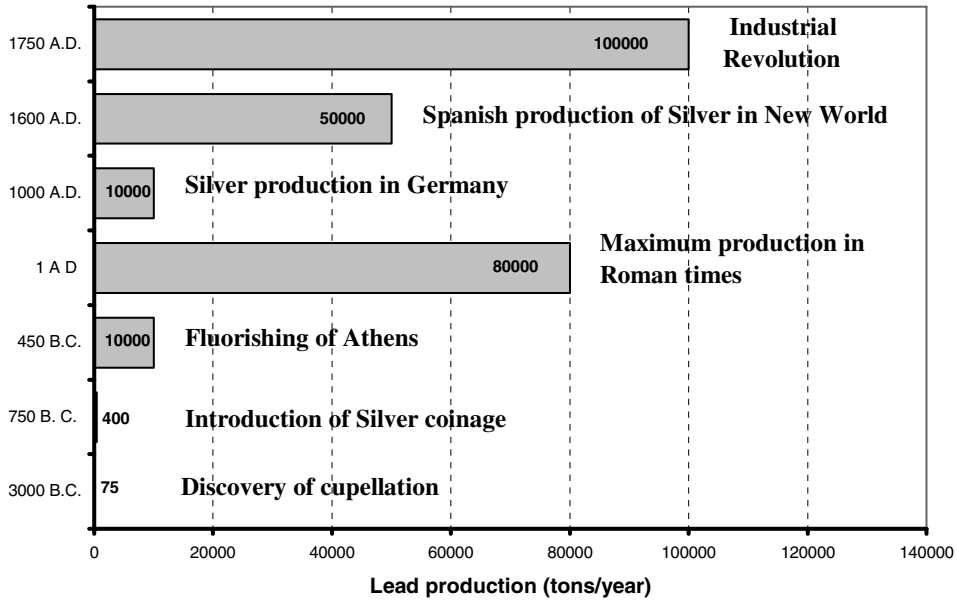


Figure 2. Milestones of world lead production (Data from Ref. 5).

the maximum splendour of the Roman Empire. Thereafter, the level of lead production slowly decreased as the Empire declined. The production of silver in Germany around 1000 A.D. gave a new boost to lead production as did the Spanish production of silver in the New World several centuries later. At this point the total world production reached a value of 50000 tons/year, practically half of the maximum level in Roman times, but the increase continued beyond this previous maximum level and reached 100000 tons/year at the beginning of the Industrial Revolution.

During this time lead production was strongly linked to silver production; about 400 parts of lead were produced as a by-product for each part of silver [5]. The demand for lead during the Industrial Revolution increased sharply and lead was not only obtained as a by-product from silver production but was independently mined and smelted. Indeed, since the beginning of the 19^{th} century the metal obtained in this way has constituted a significant part of the total lead production.

Mining production increased during the 19^{th} and 20^{th} centuries and reached a level of about 3×10^6 tons in the year 2000. However, after reaching a maximum around 1970, lead production has undergone a slow but steady decline. This is due in part to the fact that recycling is increasing and, as a result, about 50% of the 6×10^6 tons produced in the world in the year 2000 were obtained from recycled scrap lead products [6]. This recycled lead is normally known as *secondary lead*.

The technology employed to recover lead from its ores was known by different prehistoric cultures. Even though other sources could be used, the pre-eminent ore was the argentiferous galena, from which lead and silver were obtained.

Shafts and galleries were used to carry out underground extraction and illustrative examples of these remain in Laurion [7], and in Rio Tinto [1] and Cartagena [8], Spain. After extraction the ore was crushed, milled, sieved and washed in order to be concentrated. After this step, which was probably unnecessary for highly pure galena, the ore was ready for the following step, smelting, which was performed in two stages: roasting in air and heating the resulting lead(II) oxide with carbon to yield impure lead.

The small amount of available evidence for these last two operations in ancient mines suggests [7] a rough and ready undertaking that might have taken place in a simple construction. This structure was probably temporarily erected in elevated areas of the mining complex, and later dismantled. Similarly, in the Andean region of South America before the arrival of Spanish, and in several places even after, basic earthenware pots were placed on the side of the mountains to be oxygenated by the wind and used as furnaces (huayras) [9].

The operations were relatively simple from a metallurgical point of view because lead melts at 327 °C and its oxide can be reduced in charcoal or wood fires at temperatures close to 800°C.

In this process a proportion of the galena is initially oxidized. Even though lead(II) sulphate can be formed [8, 10], lead(II) oxide is the main product, especially near the stronger oxidizing point:

$$2PbS + 3O_2 \longrightarrow 2PbO + 2SO_2 \quad (1)$$

the lead(II) oxide and the unoxidized galena react to give Pb

$$2PbO + PbS \longrightarrow 3Pb + SO_2 \quad (2)$$

and the oxide is reduced with carbon

$$3PbO + 2C \longrightarrow 3Pb + CO + CO_2 \quad (3)$$

Although the operation is simple, it does suffer from some inherent problems. First of all, the proportion of lead recovered from the ores is low due to the inefficiency of the process. A significant quantity of lead remains in the slag. This meant that only ores with a very high lead content were used in ancient times and, consequently, today some early slags are exploited using modern methods. The other main problem is an environmental issue; the lead that is lost as aerosol fumes and the SO_2 that is liberated are both pollutants.

The impure lead obtained in this way is called lead bullion and contains silver, gold and other metals such as copper or zinc. Pure lead was obtained using the cupellation process; when lead bullion is melted and heated in a shallow furnace and exposed to a blast of air at about 1000 °C, lead and the other metals are oxidized while silver and gold remain as metals which enables the lead to be separated. The lead oxides formed in the process are skimmed off regularly and are used to prepare lead compounds or re-smelted to obtain lead.

The production of lead since Antiquity and the cumulative release of lead into the air has given rise to an environmental problem [5, 11, 12]. Although mines initially operated on a small scale, lead emissions increased through the uncontrolled smelting of large quantities of ores in open spaces, the introduction of large furnaces during the 16th century and the development of manufacturing during the Industrial Revolution. At the beginning of this period large amounts of relatively coarse lead ore dusts together with lead fumes were emitted into the atmosphere due to poor furnace designs and inefficient smelting procedures. These designs and procedures were progressively improved and, as a consequence, economically valuable lead aerosol smelter fumes were also increasingly recovered. The estimated fraction of wasted lead in ores from 1750 to 1880 may have been about 2%, between 1880 and 1920 that loss may have been reduced to about 0.5% and in 1970 probably to 0.06% [11].

The Pb analysis of different environmental archives in the Northern Hemisphere, such as polar ice in Greenland [11, 13], lake sediments in Sweden [14, 15] and peat bogs in Spain [16], all revealed that ancient mining activity, particularly during the Roman Empire, polluted the middle troposphere on a hemispheric scale. This pollution occurred long before the Industrial Revolution. The significance of lead emissions during this period and during the subsequent global industrial development can also be determined. Analysis of the data shows, in the latter case, a recent decrease in the Pb levels and this corresponds to the phasing out of leaded gasoline.

Due to their location, some of these archives are better able to reveal specific information about particular periods; for example, the results from Swedish lake sediments [15] show an increase in lead pollution at around 1200 A.D., which corresponds to the expansion of silver mining in Europe, particularly in Germany, after 1000 A.D.

In the Southern Hemisphere, the lower Pb content in equivalent environmental archives, for example, in the Antarctic snow when compared with the Arctic, is a result of the lower intensity of emissions [11]. Recent studies on lake sediments from the Bolivian Andes [9] show the influence not only of the Spanish but of the Tiwanaku (1000 to 1200 A.D.) and Inca peaks of production (1400-1545 A.D.) on the Pb content of the sediment.

1.3. **The historical evolution of lead poisoning.**

Lead is a normal constituent of the earth's crust and it is harmless if undisturbed but highly toxic once mined and transformed for human use.

The adverse health effects of this element are well known today as it is one of the most widely studied toxic substances (Chapter 4). In spite of this, lead poisoning is still the most common environmentally caused disease in the USA today [17].

Lead toxicity has been recognized since Antiquity even though exposure to the metal has varied significantly throughout history. At first, this exposure was only a problem for the workers that directly mined or worked the metal; later, mainly during the Roman Empire, lead was extensively used in everyday life and this led to more widespread exposure. Later still, the cumulative process of mining and use meant that the element became widely dispersed in our environment, consequently increasing the risk of exposure for all forms of life on the planet.

The significant increase in world-wide production since the Bronze Age was only possible because of the large number of workers employed to mine, smelt and refine the metal. It is estimated [4] that, on average, about 80000 miners were occupationally exposed to lead each year in the Roman Empire. This figure could reach an average of 140000 per year if one includes the craftsmen who used lead in various diverse industries. Taking into account that in lead mines or metallurgy an average working life of ten years may have been normal, Nriagu [4] suggested that over 20 million people were occupationally exposed to lead from remote antiquity to around the fall of the Roman Empire.

Despite the large number of workers in contact with the element, literary records from Roman times do not mention any widespread incidence of occupational diseases during that period. Perhaps this is because the literature has not survived or because doctors paid little attention to the people exposed to the hazards, namely slave workers or lowly craftsmen.

The daily use of lead increased sharply during the Roman Empire, as mentioned above. Lead vats, tanks, cooking utensils and pots, urns and vessels were widely used and lead pipes formed the basis for the water-carrying system for towns and houses. During this period lead was present in people's diet because of the slow dissolution of water pipes or storage tanks; other sources came from contamination or adulteration during the preparation of food and drink by the release of the metal from lead, bronze or pewter utensils, from lead-glazed pottery and also by the use of preservatives, colorants, condiments or seasonings containing lead.

Concentrated grape syrup or *sapa* was probably the most significant source of lead from this last group [18, 19]. Sapa was elaborated by boiling unfermented grape juice until it was reduced to about a third of its original volume in lead-coated pots. In this process, the acidity of the juice caused the formation of lead(II) acetate, "sugar of lead", and other lead compounds. Sapa

Table 2
Estimated lead intake during the Roman Empire

Population/ /Lead source	Lead level in source	Daily intake	Lead absorbed (Range) μg/day
The Aristocrat			
Water	50 (50-200) μg/L	1.0 L	5 (5-20)
Wines	300 (200-1500) μg/L	2.0 L	180 (120-190)
Foods	0.2 (0.1-2.0) μg/g	3000 g	60 (30-600)
Miscellaneous			5
Total			*250 (160-1520)*
The Plebian			
Water	0.5 (0.5-5) μg/L	2.0 L	0.1 (0.1-1.0)
Wines	50 (50-400) μg/L	1.0 L	15 (15-120)
Foods	0.1 (0.1-1.0) μg/g	2000 g	20 (20-200)
Total			*35 (35-320)*
The Slave			
Water	50 (50-200) μg/L	2.0 L	5 (5-20)
Wines	5 (1-10) μg/L	0.75 L	1.1 (0.2-2.0)
Foods	0.05 (0.05-0.5) μg/g	1000 g	5 (5.0-50)
Miscellaneous			5
Total			*15 (15-77)*

(Adapted from Ref.18)

was added to wine as a preservative and was also used to sweeten foods. The high lead concentration in sapa could have brought about a high lead concentration in wine.

The consumption of wine by the Roman aristocracy was rather high and, consequently, one can make a conservative estimate for the lead intake of 180 μg/day for a member of this social class; when added to a possible 60 μg/day from contamined food and other marginal sources, including drinking water, this gives the significant amount of 250 μg/day. Lower social classes had a poorer diet and as a result were less affected by the metal. Estimates of daily consumption and daily lead intake [18] by an average aristocrat, plebian and slave during the Roman Empire (on the basis of absoption factors of 0.1, 0.3 and 0.1 for water, wine and food, respectively) are given in Table 2. Even if the estimates are out by a factor of 2 to 5, there is strong evidence that a large number of Roman aristocrats were poisoned by lead due to the ingestion of food and wine.

The aristocrats, the "masters", suffered a wide range of symptoms associated with lead poisoning [18, 19]. Headaches, insomnia, jaundice and diarrhoea are typical in the early stages. Severe stomach and abdominal pains

(colic), pains in joints (gout) and extreme, even complete, constipation caused by the paralysis of the intestinal tract follow. Next come severe central nervous system disorders, including deafness, blindness, paralysis and insanity. Miscarriage and stillbirth were effects suffered by women.

During the first century A.D., Musonius (cited by Nriagu, Ref. 18) wrote: *That masters are less strong, less healthy, less able to endure labour than servants; countrymen more strong than those who are bred in the city, those that feed meanly to those who feed daintily; and that, generally, the latter live longer than the former. Nor are there any other persons more troubled with gouts, dropsies, colics, and the like, than those who, condemning simple diet, live upon prepared dainties.*

This general lack of good health was normal in aristocratic circles during this period. All of these phenomena that affected the ruling class may have influenced the vitality of the Empire, thus increasing its internal weakness [18, 20]. Other factors including economic factors arising from overexpansion and decentralization may also have contributed, possibly to a great extent, to the gradual transformation and decline of the Empire [17].

Although lead was still widely used for industrial, domestic and medicinal purposes after the Roman Empire, lead poisoning is almost unheard of in literature from the Middle Ages but reappeared in the 16th century [21], when Paracelsus describes "miner's disease"

Cases of lead poisoning linked to the consumption of sweet wine or food continued during the 16-18th centuries with severe epidemics in central Europe and later in America.

In 1697 the German physician E. Gockel published a book [19] describing a wine disease in the city of Ulm caused by sour wine sweetened with litharge. This book linked lead with a serious and sometimes fatal colic suffered by wine consumers. One year before the publication of the book, Gockel described his findings to Duke Eberhard Ludwig, who issued an edict banning all lead-based additives. As Eisinger [19] underlines, this edict may well have been the first consumer-protection legislation targeting a specific toxin.

Forty years earlier, S. Stockhausen [19], another German physician, linked lead with a common ailment in the mining towns in the Harz Mountains in Germany. He deduced that only those exposed to lead dust or vapours would fall ill. Stockhausen wrote a detailed description of the symptoms and this helped Gockel to recognize lead as the common source of both diseases.

In 1767 Sir George Baker [19, 21] published a treatise linking the so-called Devonshire colic with the contamination of cider with lead the source of which was the weights used to crush the apples.

Ocupational lead poisoning continued during the 18th, 19th and the first half of the 20th century. Classical manifestations of clinical poisoning such as anaemia, encephalopathy, colic, joint and muscle pain and kidney disorders were found in lead industry workers. Additionally, miscarriage, decreases in

fertility, risk of stillbirth and mortality of newborn children were described as symptoms in women working in lead industries [21].

Another source of lead poisoning was the use of lead-based cosmetics. This use in ancient times was mentioned above and, as recently described [22], continued until the 20th century. During the 16th century women painted their faces with a mixture of white lead and vinegar. White lead was occasionally mixed with mercury(II) chloride to peel the skin while an ointment of lead sulphate was used to remove freckles.

The use of lead-based cosmetics continued during the 17th and 18th centuries, with lead(II) carbonate the major component of a popular face powder. Diseases caused by lead cosmetics were well known among the medical community and reports of lead poisoning appeared in the newspapers during this time.

The ascent to the throne of Queen Victoria in Great Britain in 1837 marks the beginning of a period of decline in the use of cosmetics, which were mainly relegated to the theatre, where cases of lead poisoning were reported. In America, in the post-civil war period the leaded white face again became fashionable and new cases of lead poisoning were known. However, the introduction of new and alternative active agents significantly and progressively reduced the use of lead-based cosmetics during the 20th century and today they have practically been removed from the market.

Another source of lead poisoning comes from lead-based paints. This type of paint was largely used in the USA when the economy of the country was mainly agrarian and rural, with a peak during the 1920s [23]. Most lead paints still exist as a thin mass on the walls and structures of older buildings and when poorly maintained, these layers can decay and release lead from their surfaces in the form of dust. Due to its sweet taste, young children can be tempted to eat paint chips and this can have severe health consequences. In adittion, the unsafe removal of these materials releases a lead-containing dust, which constitutes a significant contribution to the presence of lead in the environment.

The contribution of lead paints, although significant, can be considered a lesser threat than the introduction of TEL as an additive in gasoline. This new development happened in the first half of the 20th century and had a marked effect on the environment, converting lead poisoning into a world-wide concern.

The toxic effects of TEL were evident shortly after industrial production started. This was in part due to General Motors' interest in launching leaded gasoline onto the market as soon as possible; production started in September 1923, before proper ventilation had been installed and before safe operating procedures had been developed, causing an occupational lead poisoning problem which lead to several deaths and also to many illnesses in production plant workers.

These facts, together with the negative comments in the press which followed, led to the belief that TEL-containing gasoline itself was a health

hazard. On May 1st 1925, the sale of leaded gasoline was suspended until its potential public health hazards could be assessed.

A committee composed of scientists, public health officials and industrial representatives was set up to investigate the safety of the product. At a committee meeting, R. Kehoe, who represented the industry, tried to play down the risks, while A. Hamilton, supported by other scientists, pointed out that the risk, if there was one, would spread to the whole population. In spite of these concerns the meeting did not lead to any conclusion and a new committee was created to study the problem further. As this committee concluded there was no evidence that TEL presented a hazard to the community, in May 1926, after a year of moratorium, leaded gasoline was back on the market [2, 21, 24, 25].

After this date, TEL production continuously increased as the sale of leaded gasoline also increased. However, public concern over the introduction of lead into gasoline continued, in particular when people began taking into account the increasing number of automobiles and the cumulative effect of the well-known toxic lead inorganic compounds which were formed in the engine and liberated to the environment.

The controversy between environmental and health advocates and industry representatives iniciated in 1925 continued for several years and the research which was being carried out to support the confronted positions was essential for the evolution of the concept of lead toxicity, and also contributed to define the concept of lead poisoning and consequently to prevent it. The debate had two well-defined positions, based on different methods and conclusions, and these were presented by R. Kehoe and C. Patterson [21, 24, 25].

Kehoe defined lead poisoning strictly in clinical terms, accepting the term poisoned only when the blood lead level surpassed the 80 μg/dL, whereas a certain concentration of lead in blood, about 20 μg/dL, was considered "natural" and "normal" [21]. This position, shared by other researchers, was supported by data obtained from lead-contaminated laboratories, which as a result raised the baseline measurements of all their samples. In addition, particular attention was not paid to the control subjects selected for the studies, who in many cases had previously been exposed to lead.

By using a very clean laboratory to study uncontaminated samples, Patterson [26] showed that technological activity had raised the lead body burdens of modern humans by about 100 times compared to pretechnological man. He therefore concluded that the "natural" levels should be those of uncontaminated prehistoric man, and that any other levels are indicative of lead poisoning, even though this aspect was not evident from a clinical analysis. The idea that even these slight effects were unacceptable in terms of health gathered progressive scientific support and finally led to the removal of TEL from petroleum products.

As mentioned above, the use of TEL was banned in the USA (this action brought about a decline in blood lead values in children and adults by 80% [25]) and this elimination is being progressively adopted in other countries.

2. OCCURRENCE AND ISOLATION

2.1. Occurrence

Although lead can be found in nature as the pure element, this is extremely rare and it is usually present as Pb(II) in deposits with different origins. In these sources it is combined with other elements such as sulphur and oxygen in a variety of minerals that have a wide range of compositions.

The compositions of some of these materials are shown in Table 3 [1, 17, 27] and it is clear that the presence of sulphides, in some cases incorporating selenium, is rather relevant. Some of these minerals that contain a stoichiometric amount of silver have been widely used since ancient times to coproduce silver an lead, e.g., plumbojarosite in Spain [1, 9], whereas other minerals incorporate silver in varying amounts, ranging from 0.5 to 5 mg/g [1]. In particular, galena (Fig. 3), by far the most important lead mineral, commonly contains microscopic bodies of silver-rich minerals that can give a silver concentration of 8 mg/g; this phenomenon could also explain the use of this mineral in the production of silver since ancient times.

Besides galena and other associated mixed sulphides, there are several minerals such as cerussite ($PbCO_3$), anglesite ($PbSO_4$), litharge/massicot (PbO) and minium (Pb_3O_4), that are regularly used in the production of lead. Some of the most common impurities found in lead minerals are zinc, copper, arsenic, tin, antimony, silver, gold and bismuth.

Figure 3. Argentiferous galena (Courtesy of the Luis Iglesias Museum from the University of Santiago de Compostela, Spain).

Table 3
Representative lead minerals

Mineral	Composition	Mineral	Composition
Sulphides		*Others*	
Galena	PbS	Clausthalite	$PbSe$
Geocronite	$Pb_5(Sb,As)_2S_8$	Altaite	$PbTe$
Beegerite	$Pb_6Bi_2S_9$	Penroseite	$(Ni,Cu,Pb)_2Se_2$
Bournonite	$PbCuSbS_3$	Litharge	PbO
Meneghinite	$CuPb_{13}Sb_7S_{24}$	Massicot	PbO
Boulangerite	$Pb_{2-5}Sb_{2-4}S_{5-11}$	Minium	Pb_3O_4
Cosalite	$Pb_2Bi_2S_5$	Cerussite	$PbCO_3$
Selenocosalite	$Pb_2Bi_2(S,Se)_5$	Anglesite	$PbSO_4$
Kobellite	$Pb_2(Bi,Sb)_2S_5$	Wallenite	$PbMoO_4$
Selenokobellite	$Pb_2(Bi,Sb)_2(S,Se)_5$	Crocoite	$PbCrO_4$
Franckeite	$Pb_5Sn_3Sb_2S_{14}$	Pyromorphite	$Pb_5(PO_4)_3Cl$
Cylindrite	$Pb_3Sn_4Sb_2S_{14}$	Vanadinite	$Pb_5(SO_4)_3Cl$
Jamesonite	$Pb_4FeSb_6S_{14}$	Plumbogummite	$PbAl_3(PO_4)_2(OH)_5{\cdot}H_2O$
Semseyite	$Pb_9Sb_8S_{21}$	Tsumebite	$Pb_2Cu(OH)_3(PO_4){\cdot}3H_2O$
Zinkenite	$Pb_6Sb_{14}S_{27}$	Percyclite	$Pb_3(CO_3)Cl_2$
Plagionite	$Pb_5Sb_8S_{17}$	Phosgenite	$Pb_2Cl_2CO_3$
Nagyagite	$Pb_5Au(Te,Sb)_4S_{5-8}$	Boleite	$Pb(Cu,Ag)Cl_2(OH)_2{\cdot}H_2O$
Wittite	$Bi_6Pb_5(Se,S)_{14}$	Argentiam plumbojarosite	$(Pb,Ag)Fe_{3-6}(SO_4)_{2-4}(OH)_{6-12}$
Benjaminite	$Pb(Cu,Ag)Bi_2S_4$		
Fizelyite	$Pb_3Ag_2Sb_8S_{18}$	Plattnerite	PbO_2
Ramdohrite	$Pb_3Ag_2Sb_3S_{13}$		
Andorite	$PbAgSb_3S_6$		
Hutchinsonite	$(Pb,Tl)_2(Cu,Ag)As_5S_{10}$		
Seligmannite	$PbCuAsS_3$		
Marrite	$AgPbAsS_3$		
Lengenbachite	$Pb_6(Ag,Cu)_2As_4S_{13}$		
Diaphorite	$Pb_2Ag_3Sb_8S$		
Freieslebenite	$Pb_3Ag_5Sb_5S_{12}$		
Owyheenite	$Pb_5Ag_2Sb_6S_{15}$		
Schirmerite	$PbAg_4Bi_4S_9$		

(Adapted from Refs. 1, 17 and 27)

These minerals are scattered in deposits that can be classified depending on their origin [27], namely: (a) hydrothermal vein, impregnation and replacement deposits; (b) volcanogenic sedimentary deposits and (c) hydrothermal or marine sedimentary deposits. As far as mined deposits are concerned, mixed lead-zinc ores are currently the most important and are certainly more important than those deposits that essentially contain lead ores; the remainder are zinc, copper-zinc and other systems from which lead is obtained as a by-product.

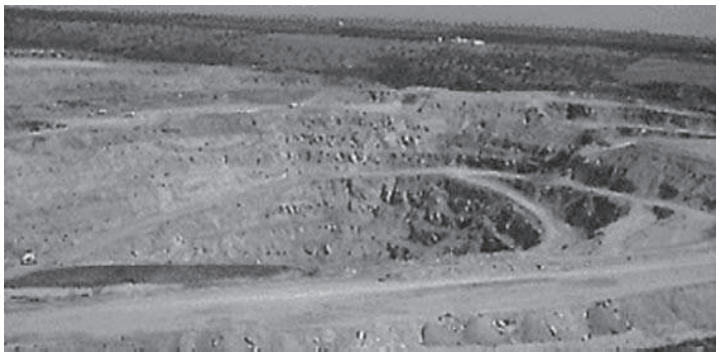


Figure 4. The Los Frailes open-pit mine (Aznalcóllar, Spain) with a design capacity of 125000 tons/year of zinc, 48000 tons/year of lead, 4700 tons/year of copper and 90.8 tons/year of silver (http://www.mining-technology.com).

In order to exploit these deposits the size, shape and quality of the ore body must be determined and then the best way to mine it can be selected.

Underground mines like the previously cited are now in operation together with surface mines like shown in Fig. 4. Unfortunately, this mine made the headlines because of the failure of the exploitation´s tailings dam on April 25, 1998. As a result, 4-5 million cubic meters of toxic tailings slurries and liquid were released into a nearby river. The slurry wave flooded several thousand hectares of farmland and threatened the Doñana National Park, a UN World Heritage Area.

Although lead mining production today is spread all over the world, the main producers of lead minerals are China, Australia, the USA, Peru, Canada and Mexico, which together account for three quarters of the world´s mining production.

Besides these natural geochemical sources, lead is widely distributed as an environmental pollutant due to the cumulative production and use as mentioned previously and this aspect will be described in detail later (Chapter 4).

2.2. Production.

2.2.1. *Primary production.*

Lead ores in deposits are usually present with other minerals and rocks. These ores cannot be smelted immediately after they have been mined, so the first step in the production process is to concentrate the ore in order to obtain a higher concentration of lead. In the past a method based on flowing water was used to achieve this, but today the technique used is froth flotation. The ore is first crushed and ground into fine particles and then it is suspended in water to obtain a pulp. Frothing agents are subsequently added and air is bubbled through the pulp under agitation; finally, a stable froth containing the lead mineral particles rises to the surface and is skimmed off.

The next step in the process involves extracting the lead. Detailed descriptions of the different methods in use today can be found in references 6, 27 and 28; only some of the most essential aspects are described here. The traditional two-stage process, which is based on roasting to obtain the oxide and heating in a blast furnace to reduce the oxide with carbon [Eq. (1-3)] is still used today, even though numerous technological improvements have been incorporated to reduce pollution and to increase the yield. The end result is known as lead bullion, which is lead that mainly contains metallic impurities, and this is tapped off from the bottom of the furnace and subsequently refined.

As mentioned above, lead and zinc usually occur together in ores. In this case, the froth flotation technique is further complicated by the use of depressant agents to stop zinc sulphide being incorporated into froth; lead and zinc ores can then be separated and treated. The Imperial Smelting Process is an alternative method that can be used for the simultaneous production of zinc and lead from this type of ore. It is a variation of the blast furnace technique, but the lead-zinc ore is directly added to the furnace and the liquid lead bullion tapped conventionally from the bottom after reduction and the metallic zinc is distilled off as a vapour.

Despite the fact that technological improvements have been introduced into the traditional two-stage method, it is still inefficient and harmful to the environment. This drawback has brought about the introduction of a new process based on direct smelting, in which there is only a single step from the sulphide to the metal. This approach makes it unnecessary to produce lead oxide in a preliminary step in order to reduce it to lead afterwards. The Kivcet process, developed specifically in the USSR to treat lead ores with high zinc contents, and the QSL, Isasmelt and Outokumpu processes are all examples of this innovation; detailed descriptions of these techniques are included in references 6, 27 and 28. Together, these direct techniques and the Imperial Smelting Process only account for 20% of lead production, with the conventional two-stage methods accounting for 80% [6].

Lead bullion contains other elements (Cu, As, Sb, Sn, Zn, Au, Ag, Bi, ...) that must be separated in a subsequent refining process and this can be either pyrometallurgical or electrolytic. When pyrometallurgical methods are used, copper is the first metal to be removed. The bullion is melted and held just above its melting temperature, at which point the copper rises to the surface and can be skimmed off. Sometimes sulphur is added in order to facilitate the removal of copper.

Arsenic, antimony and tin are more reactive than lead and can be removed by preferential oxidation. In the softening process, a name derived from the fact that these elements are standard hardeners for lead, lead is melted and stirred with a blast of air. The impurities are oxidized and form a molten slag, which is then skimmed off. In the alternative Harris process, a molten flux of sodium hydroxide and sodium nitrate is employed; after the molten bullion and the flux

are stirred, the impurities are incorporated into the alkali flux as sodium antimonate, arsenate and stannate, and if zinc is present it is also incorporated as zinc oxide. The flux and lead can then be separated and the elements extracted from the flux.

Silver and gold were separated in ancient times by cupellation; modern methods such as the Parker process or Port Prize Process extract these metals from lead melted with zinc. The silver and gold form a floating alloy with zinc and this can be separated. The zinc can then be removed by vacuum distillation. This technique is also used to remove trazes of zinc still present in lead.

Bismuth, the remaining element, can be removed by adding a calcium-magnesium alloy to the molten lead; this alloy incorporates bismuth and rises to the top of the melt where it can be skimmed off. However, bismuth is normally separated by electrolytic refining using the Betts process, which was developed at the beginning of the 20th century. In this process large cast anodes of bullion and thin cathodes of high purity lead are used in a cell containing acid lead fluosilicate as the electrolyte. When an electric current is applied, the anodes of impure lead are dissolved and pure lead is deposited on the cathode. The use of a sulphamate electrolyte as an alternative was proposed in Italy in 1950 and seems to be equally efficient.

The selection of a complete and appropiate refining scheme enables the production of bulk quantities of lead of 99.99% purity and, for special purposes, additional processing can give lead of 99.9999% purity.

2.2.2. Secondary production.

A significant proportion of the lead produced in the world each year has been recycled and is known as secondary lead. Most of this lead comes from scrap lead-acid batteries and, to a lesser degree, lead pipe, sheet and cable sheathing. Whereas these latter sources are clean and can be easily re-melted and if necessary refined, a meticulous sequential process is required to obtain lead from batteries.

Once the acid has been collected, the batteries are broken open and the case fragments, metal grids and poles are separated to be independently recycled. The paste inside each unit, which contains lead oxide, hydroxide, carbonate and sulphate, is usually treated to remove the suphur and smelted with coke in a blast furnace, although today rotary furnaces or furnaces working on a semi-continuous basis are alternatives to the blast furnace.

The lead recovered from batteries is generally used to produce new battery alloys, although it can also be refined to give lead that is suitable for any application by using the techniques outlined above.

The production of secondary lead is now significant in several countries, for the most part in highly industrialized countries. The top lead producers and recyclers of 2003 are shown in Table 4 [6]. Note the difference between these countries and the producers of lead minerals mentioned above. Countries not

Table 4
Largest lead producers and recyclers in the year 2003

	Producers (Thousand tons)	Recyclers (Thousand tons)
China	1533	
USA	1338	1098
Germany	352	222
UK	338	176
Australia	304	
Japan		190
Italy		153

(Data from Ref. 6)

endowed with mineral deposits must import ore, impure lead or scrap lead to produce refined lead, which can also be produced from their own scrap.

From a global perspective, the importance of secondary lead production is increasing progressively whereas mining production is slowly decreasing. The evolution of world-wide lead production compared to mining production over recent years is shown in Figure 5 [6].

The geographical distribution of world-wide lead production is changing. The evolution of production during the last few years in the five big geographical areas can be seen in Figure 6 [29, 30]; note the decreasing trend for production in Europe and, to a lesser extent, America, and the increasing trend for production in Asia.

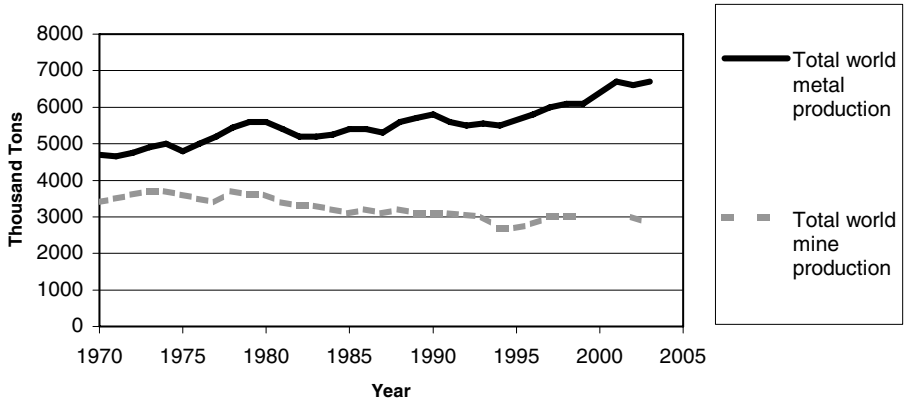


Figure 5. Recent evolution of the global total and mined lead production (Adapted from Ref. 6).

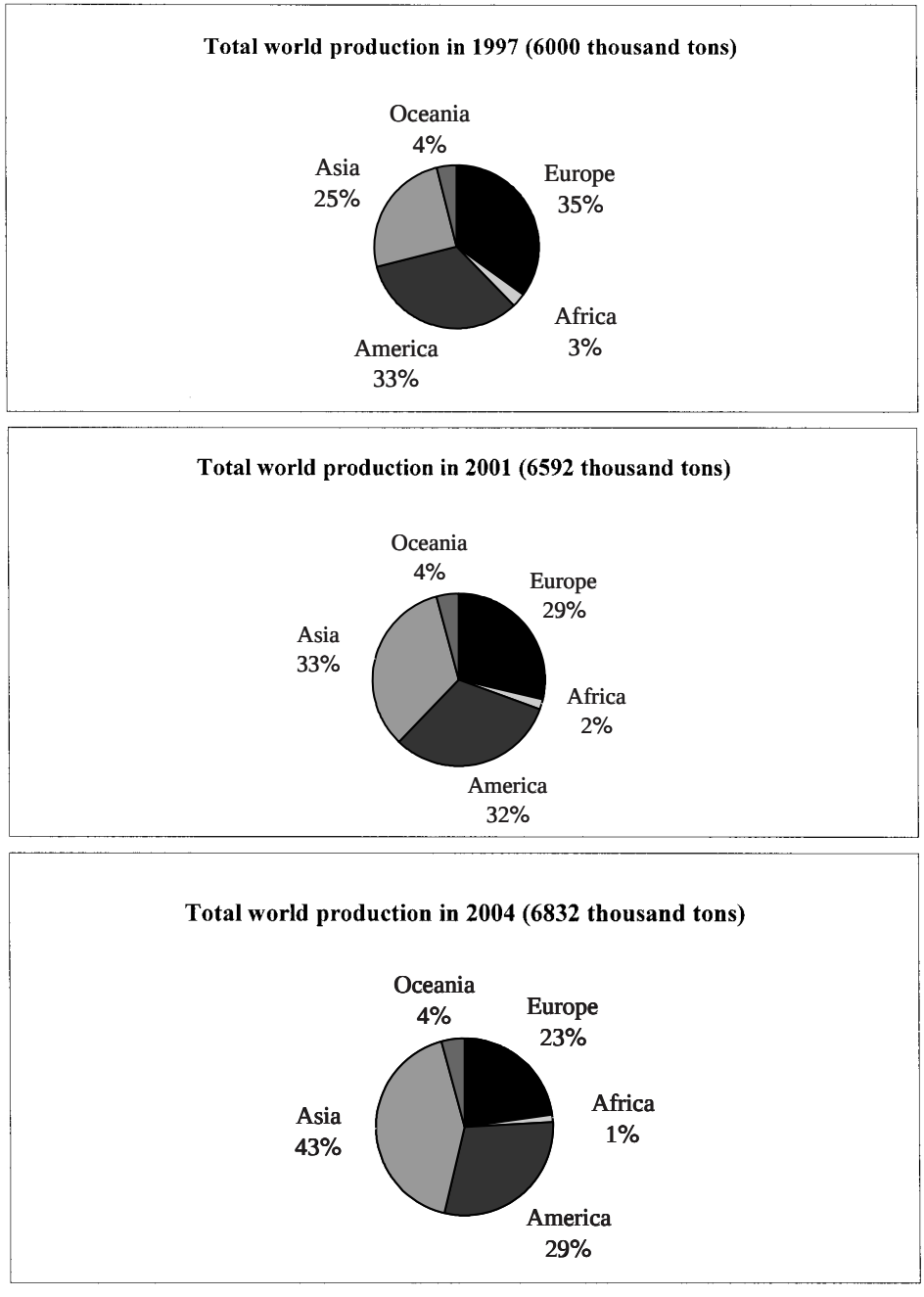


Figure 6. Evolution of the geographical distribution of world production in recent years. (Data from Refs. 29 and 30).

3. PROPERTIES

The element is a bluish-white lustrous heavy metal from group 14 of the Periodic Table. Lead crystals are face-centered cubic and have a short lead-lead distance of 3.49 Å.

3.1. Atomic properties

The main atomic properties of lead [31, 32] are shown in Table 5.

The element has four common stable isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb) and several radioactive isotopes. The most significant data for the stable isotopes and the radioactive isotopes that have half-lives longer than 10 hours are shown in Table 6.

The isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb are the stable end products of natural radioactive decay sequences and the ratio of these isotopes is different for each source in the environment depending [17] on the radioactive source from which the lead was derived and the relative decay rates of the radioactive elements: ^{206}Pb comes from ^{238}U ($t_{1/2} = 4.5 \times 10^9$ years); ^{207}Pb comes from ^{235}U ($t_{1/2} = 0.70 \times 10^9$ years) and ^{208}Pb comes from ^{232}Th ($t_{1/2} = 1.40 \times 10^{10}$ years). The ^{206}Pb/^{207}Pb [15, 16] and the ^{207}Pb/^{208}Pb [11, 26] ratios have been used to determine lead in environmental archives.

Of these stable isotopes ^{207}Pb is the only magnetically active. The characteristics of this isotope [17, 33] (spin I = ½, a fairly natural abundance of 22.6%, an appreciably high NMR receptivity 11.9 times greater than that of ^{13}C) are the basis of ^{207}Pb NMR spectroscopy, which has become a valuable and routine tool in many fields of lead chemistry.

Table 5
Atomic properties of lead

Atomic number		82
Electronic structure		[Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$
Relative atomic mass[a]		207.2
Ionization energy[b]/kJ mol^{-1}	I	715.4
"	II	1450.0
"	III	3080.7
"	IV	4082.3
Atomic radius[a]/pm		175
Pauling Electronegativity[a]		2.33
Allred Electronegativity[a]		1.55
Absolute Electronegativity[a]/eV		3.90

[a] Ref. [32]
[b] Ref. [31]

In the specific field of lead(II) coordination compounds, the Pb chemical shift provides an excellent measure of the type of donor atom bound to lead [17]. The lead resonance tends to shift upfield as the electronegativity of the ligand donor atom increases. Such a change is observed, for example, on changing from S to N or O, with equivalent donor atoms being sensitive to the lead coordination number [17]. Indirect detection methods such as HMQC $^{1}H/^{207}Pb$ can be applied [34] to study dilute aqueous solutions and could be useful to characterize lead-binding sites in complex systems.

The environment of the Pb atom in diorgano or triorganoderivatives of Pb (IV) in solution was also investigated using this technique [35, 36]. A number of examples of the contribution of this technique to the structural knowledge of lead organoderivatives are outlined in Chapter 3.

Solid state ^{207}Pb NMR spectra can be obtained for static samples or by MAS and CP MAS techniques. In general, the spectra are characterized by large chemical shift anisotropies and by large distributions of chemical shifts due to the strong effects that even small changes in bond angles and distances in the Pb environment can have on the chemical shifts [33, 37].

3.2. Physical properties

Lead has physical properties common to other metals: it has a metallic lustre with shiny freshly cut surfaces, a high density, a low melting point, it is a conductor of electricity and heat and is soft, ductile and malleable.

Table 6
The stable and radioactive[a] isotopes of lead[b]

Nuclide	Atomic mass	Natural abundance (%)	Nuclear spin	Half-life ($t_{1/2}$)
^{204}Pb	203.973 020	1.4	0+	
^{206}Pb	205.974 440	24.1	0+	
^{207}Pb	206.975 872	22.1	1/2-	
^{208}Pb	207.976 627	52.4	0+	
^{200}Pb	199.971 790		0+	21.5 h
^{202}Pb	201.972 134		0+	53000 y
^{203}Pb	202.973 265		5/2-	2.162 d
^{205}Pb	204.974 458		5/2-	1.51×10^{7} y
^{210}Pb	209.984 163		0+	22.6 y
^{212}Pb	211.991 871		0+	10.64 h
^{214}Pb	213.999 798		0+	27 m

[a] half-life longer than 10 hours
[b] Ref. [32]

Table 7
Physical properties of lead

Density[a] /g·cm^{-3}	11.34
Melting point[a] / °C	327.5
Boiling point[a] / °C	1750
ΔH_{fusion}[b] / kJ.mol^{-1}	5.121
ΔH_{vap}[b] / kJ.mol^{-1}	179.4
Thermal conductivity[b] (300K) / W m^{-1}k^{-1}	35.2
Coefficient of linear thermal expansion[b] / K^{-1}	29.1×10^{-6}
Electrical resistivity[b] (293K) / Ohm·m	20.648×10^{-8}
Young's modulus[b] / GPa	16.1
Rigidity modulus[b] / GPa	5.59
Poisson's ratio[b] / Gpa	0.44

[a] Ref. [28]
[b] Ref. [32]

Some significant physical data [28, 32] for the element are shown in Table 7. The particularly high density is a result of a high relative atomic mass and the face-centred cubic structure with a short lead-lead distance in which it crystallizes.

3.3. Chemical properties

3.3.1. The element

The freshly cut metal rapidly loses it metallic shiny lustre in moist air due to the formation of a layer of lead(II) oxide on the surface. The oxide can further react with carbon dioxide to form lead(II) carbonate. Under normal conditions this surface layer protects the bulk of the metal against further attack. At high temperatures lead also reacts with sulphur and the halogens.

Even though pure deareated water does not attack lead, the element is oxidized by the joint action of oxygen and water to give Pb(II). The metal dissolves:

$$Pb(s) \longrightarrow Pb^{2+}_{(aq)} + 2e^-$$

and the electrons are consumed in reactions such as:

$$\tfrac{1}{2}\, O_{2(aq)} + H_2O_{(l)} + 2e^- \longrightarrow 2OH^-_{(aq)}$$

or

$$2H_3O^+_{(aq)} + 2e^- \longrightarrow H_{2(g)} + H_2O$$

in aereated water or acid media, respectively.

This corrosion reaction could produce disolution in water, which in practice is very slow and is often controlled by the reaction products, Pb(II) salts. Such salts, in general have low water solubility and can act as protective layers, a phenomenon that will be discussed later. The pH and the presence of other species can affect the solubility of these salts and therefore the solubility of lead is conditioned by the exact composition of the water.

For example, when Pb is attacked at a basic pH, the formation of PbO and Pb_3O_4 is more likely. In the presence of CO_2, these oxides are relatively soluble but are also readily converted to the less soluble lead (II) carbonate or to the basic carbonate $2PbCO_3.Pb(OH)_2$. A high concentration of carbonate ions in water, as in the case of hard water, leads to the formation of solid lead(II) carbonate, which precipitates on the inner walls of the pipes and prevents further lead corrosion. In contrast, soft water with a pH < 6.5 has a much greater corrosive effect on lead pipes. This effect can be limited by adding phosphate to the water supply to decrease lead solubility and to protect the internal walls of lead pipes.

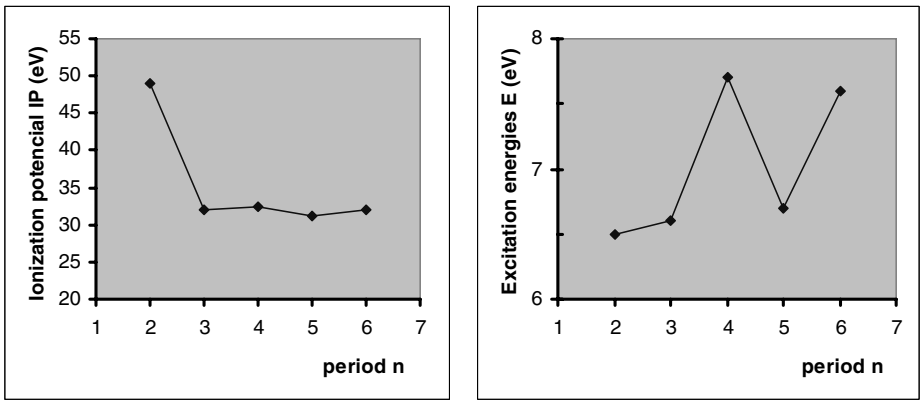
This reaction is important if lead pipes are used to carry drinking water, because although this slow attack brings about a low concentration of Pb(II), it is enough to produce chronic intoxication when the water is consumed.

In strongly acidic media, the overpotential for the discharge of hydrogen and the formation of insoluble salts as a protective layer makes lead resistant to attack by sulphuric (lead can be used for handling concentrated sulphuric acid), phosphoric and chromic acids. Acetic acid in the presence of oxygen rapidly attacks lead and produces the very soluble lead(II) acetate, which precludes the use of Pb to process or store wine or fruit juices. Hydrochloric acid produces slow attack and the oxidizing nitric acid reacts quite rapidly to give the very soluble lead(II) nitrate.

3.3.2. Oxidation states

The element has two common oxidation states, Pb(II) (electronic structure: [Xe] $4f^{14}$ $5d^{10}$ $6s^2$) and Pb(IV) (electronic structure: [Xe] $4f^{14}$ $5d^{10}$). The former dominates the inorganic chemistry of lead while organolead chemistry is dominated by the latter. In water lead is easily oxidized to Pb(II), $\varepsilon^{o}(Pb(II)/Pb) = -0.1251$ V, and Pb(IV) compounds are strong oxidizing agents, $\varepsilon^{o}(Pb(IV)/Pb(II)) = 1.69$ V, that are reduced to Pb(II). This last oxidation state is the inorganic form that is predominant in the environment and the most significant from a toxicological point of view.

The preference of lead by the oxidation state (II) in its inorganic chemistry has been traditionally attributed to the effect of the "inert electron pair", a concept introduced by Sidgwick [38] to explain the tendency of the post-lanthanide elements (Tl, Pb and Bi) to adopt oxidation states two units below the state usually adopted by the light elements of their respective groups. Thus, in Group 14 of the periodic table, carbon chemistry is dominated by the oxidation



a) b)

Figure 7. a) Experimental $ns^2 \rightarrow ns^1$ ionization potentials for Group 14; b) Experimental $ns^2 \rightarrow ns^1np^1$ excitation energies for Group 14. (Adapted from Ref. 39).

state (IV) but lead chemistry by the state (II). A certain degree of "inertia" associated with the 6s electron pair of lead(II) is supported to some extent by certain atomic parameters. It can be seen in Figure 7 that there is an increase in both the sequence of the first ionization potentials (although not shown, the sequence is very similar for the second) and the s-p orbital energy gap when one moves from the 5^{th} (Sn) to the 6^{th} (Pb) period. These increases are due to a relativistic effect [39].

However, this explanation for the stabilization of the oxidation state (II) in lead chemistry, when exclusively supported in terms of an intrinsic atomic property of the metal, is not completely satisfactory. The atoms bound to lead must play a role that can dominate the relativistic stabilization of its $6s^2$ electron pair, leading to Pb(IV) compounds in which the 6s pair is involved in the bond. Thus, as mentioned above, PbR_4 organolead compounds (Chapter 3) are usually more stable than PbR_2 ones. Furthermore, theoretical studies on relatively simple molecules reduce the relative importance of the relativistic stabilization and suggest that the $6s^2$ pair can be involved to some extent in the bonds even in Pb(II) compounds [39, 40].

The term "inert", as applied to the $6s^2$ electrons, should therefore be avoided and replaced when necessary in the context of the Pb(II) chemistry by "lone", which is a more neutral term. As described in Chapter 2, this pair can become a stereochemically active lone electron pair (SALEP) in lead(II) compounds and sometimes has a decisive influence on the arrangement of the atoms bound to the metal. The explanation for this phenomenon is, however, not without controversy (see Chapter 2).

3.3.2.1. Pb(II)

In water, Pb(II) is mainly present as $Pb^{2+}_{(aq)}$. A recent theoretical treatment [41] has provided a detailed picture of the hydrated structure, with a flexible first hydration shell of nine water ligands and a second shell in which the coordination number varies from 18 to 28 water ligands (average value: 24.6).

The water-exchange rate constant, which is a measure of the rate of substitution of the inner-sphere water ligands, is 7.5×10^9 $M^{-1}s^{-1}$ and this is towards the higher values for common cations [42], suggesting that exchange rates for other monodentate ligands in aqueous solution are also expected to be rapid.

The pK_h (K_h = hydrolysis constant) of 7.71 [42] is indicative of a certain degree of hydrolysis. For example, a 0.1 M $Pb(NO_3)_2$ solution shows a native pH of 2.4, indicating that 4.0% of the Pb(II) ions are hydrolysed. A recent study [43] of the Pb(II)/OH system in $NaClO_4$ media at 25°C reveals that at [Pb] ≤ 10μM and [OH^-] ≤ 5M the hydroxo species present are $[Pb(OH)]^+$, $Pb(OH)_2$, $[Pb(OH)_3]^-$ and $[Pb(OH)_4]^{2-}$. At higher Pb(II) concentrations, polycations such as $[Pb_3(OH)_4]^{2+}$, $[Pb_4(OH)_4]^{4+}$ (which could be formed by the association of $[Pb(OH)]^+$ [37]), $[Pb_6(OH)_6]^{6+}$ and $[Pb_6O(OH)_6]^{4+}$ have been reported.

The structure of the $[Pb_4(OH)_4]^{4+}$ cluster present in $[Pb_4(OH)_4](NO_3)_4$ was determined by X-ray diffraction and consists of four Pb atoms at the corner of a slightly irregular tetrahedron, with all the faces capped by hydroxide O atoms [44]. The cation $[Pb_6O(OH)_6]^{4+}$ was identified [44] in solution at pH ~ 8.5-11.2. The crystal structure of $[Pb_6O(OH)_6](ClO_4)_4.H_2O$ [45] shows that the cation consists of a central distorted Pb_4 tetrahedron, with an O atom at the center, and the two remaining Pb atoms capping two faces of this tetrahedron, thus defining two external tetrahedra. The six remaining O atoms from the hydroxide groups are located over the six external faces of these two tetrahedra.

Although the mononuclear ions seem to be the only significant hydrolysis products of Pb(II) under typical environmental and biological conditions, the importance of the polynuclear cations can be relevant under certain conditions [44].

Although the organometallic and coordination compounds will be discussed in later chapters, some of the more significant aspects of the simple inorganic lead compounds [31, 46] will be briefly covered below.

Lead(II) oxide, PbO. This is the oxide formed when lead is heated in air, and it is manufactured by blowing air into molten lead. Two crystal forms of the oxide are known, the *red* tetragonal form *(α-PbO, litharge)*, which is stable at room temperature, and the *yellow* orthorhombic form *(β-PbO, massicot)*, which is stable above 489°C.

The crystalline red form has a layered structure. Within each layer the O atoms form a square planar arrangement. Each four O atoms constitute the base of a square pyramid with the Pb atom (or a SALEP) at the apex (Fig. 8a); these

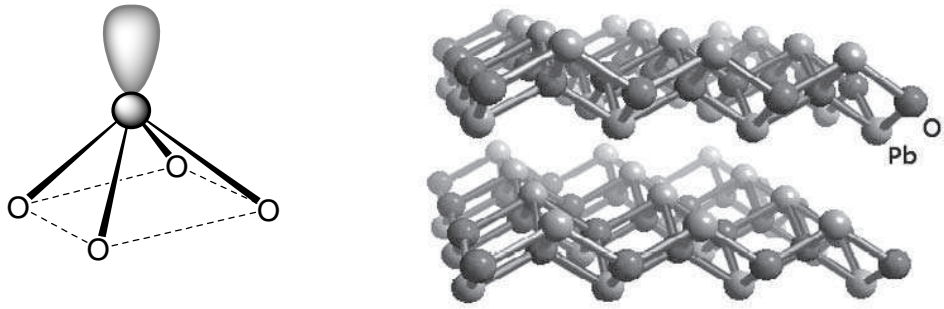


Figure 8. Structure of red tetragonal PbO (litharge) showing: *a)* a single square-based pyramid and *b)* the arrangement in layers.

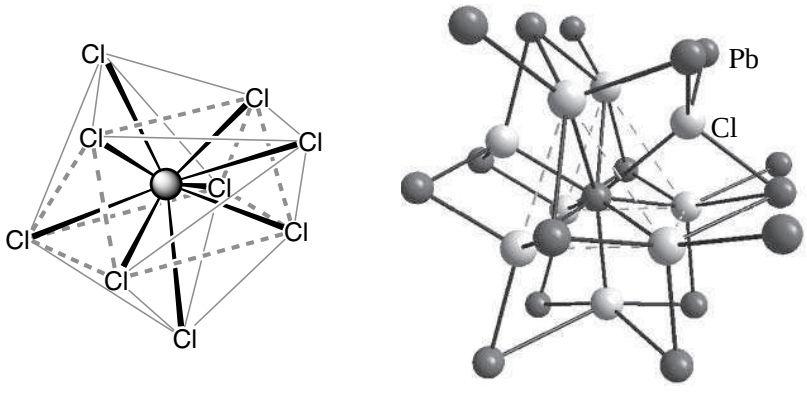
pyramids are alternately arranged with the Pb atoms above and below the O plane (Fig. 8b). The structure of massicot is similar to that of litharge, but the four equal Pb-O bond distances present in litharge are replaced in massicot by two shorter and two longer lengths.

Lead(II) oxide dissolves readily in acids and is only slightly soluble in NaOH solutions, except when they are very concentrated. The addition of alkali metal hydroxide solutions to solutions of Pb(II) salts leads to the formation of a white hydrate $PbO.xH_2O$ ($x<1$) as a precipitate. When this precipitate is heated to 100 °C it is converted into *red* PbO or *yellow* PbO if heated at lower temperatures. A pure $Pb(OH)_2$ hydroxide has not yet been prepared.

Lead(II) sulphide, PbS. This compound is found in nature as the mineral *galena* and precipitates as a black powder from solutions of Pb(II) salts when they are saturated with H_2S. It may be synthesized in a crystalline form by adding thiourea to a sodium plumbite solution.

PbS adopts the cubic NaCl-type structure, with the Pb atom in an octahedral environment. This situation is in contrast to that discussed previously for this atom in the tetragonal form as adopted by PbO at room temperature. A recent analysis [47] of the electron density, density of states and crystal orbital overlap populations suggests that the different symmetry of the electron density of Pb(II) in both structures is a direct result of the different anion-cation interaction. In PbO, the asymmetric electron density on Pb(II) is a result of the interaction of the antibonding combination of the Pb 6s and O 2p orbitals with the unfilled Pb 6p orbital. The higher energy of the S 3p orbital compared to the O 2p orbital leads to a weaker anion-cation interaction and also to the absence of coupling between the equivalent antibonding combination and the Pb 6p orbital.

Lead(II) sulphide is insoluble in deareated water but can be attacked by oxygenated water. This fact, together with the oxidation of this material by oxygen, led to the partial evolution of galena deposits in nature to form lead(II)



a) *b)*

Figure 9. Structure of $PbCl_2$ showing the tricapped trigonal prismatic arrangement around Pb.

sulphate and sulphuric acid. The solubility of PbS increases in acidic media and an additional route of attack on galena can occur in highly acidic waters. In contrast, insoluble lead(II) carbonate complexes are formed in alkaline media.

This compound is an intrinsic semiconductor and can have *n*-type or *p*-type properties depending on the stoichiometry (*p*-type semiconductor when S rich and *n*-type semiconductor when Pb rich) and/or the level of impurities.

Lead(II) selenide, PbSe. This compound is found in nature as the mineral *clausthalite* and is isomorphous with galena. PbSe is synthesized by reacting the two elements, by the action of H_2Se upon lead salts or by adding selenourea to a solution of lead(II) acetate in the presence of hydrazine. This compound adopts the NaCl structure and behaves as a semiconductor.

Lead(II) telluride, PbTe. This compound is found in nature as the mineral *altaite*. It can be prepared by reacting the elements together or by adding tellurium powder to a boiling solution of a lead(II) salt. The material behaves as a semiconductor and is a photoconductor at low temperatures.

Lead(II) fluoride, PbF_2. This is a colourless solid and it can be prepared by dissolving lead(II) carbonate in hydrofluoric acid followed by decomposition of the resulting hydrofluoride. PbF_2 has the rhombic $PbCl_2$ structure below 316 °C and the cubic CaF_2 structure above this temperature.

Lead(II) chloride, $PbCl_2$. This compound is prepared by reacting lead(II) oxide, acetate or carbonate with hydrochloric acid. In crystalline $PbCl_2$ each Pb atom is coordinated by nine Cl atoms, six of which lie at the apices of a trigonal prism and the remaining three beyond the centres of the three prism faces (Fig. 9a). Each Cl is coordinated by four or five Pb atoms (Fig. 9b).

Upon exposure to air lead(II) chloride readily forms basic chlorides such as, for example, $PbCl_2.Pb(OH)_2$, which is used as a pigment.

Lead(II) bromide, $PbBr_2$. This compound can be prepared by the same procedure as for the chloride, but using hydrobromic acid. The material has the $PbCl_2$-type structure and decomposes slowly when exposed to light, producing lead.

Lead(II) iodide, PbI_2. This can be prepared by reacting a water-soluble Pb(II) salt with hydroiodic acid or with a soluble metal iodide. The compound has the CdI_2 structure.

All the halides are sparingly soluble in cold water but their solubility increases on heating.

Lead(II) carbonate, $PbCO_3$. This compound occurs in nature as the mineral *cerussite*. It is prepared by passing CO_2 through a solution of cold dilute lead(II) acetate.

A basic lead(II) carbonate, $2PbCO_3.Pb(OH)_2$, known as white lead and found in nature as *hydrocerussite*, can be prepared by reacting lead(II) acetate with carbon dioxide in the presence of air.

Lead(II) nitrate, $Pb(NO_3)_2$. This compound is prepared by dissolving Pb or PbO in hot dilute nitric acid. The material is very soluble in water and is widely used to prepare other Pb(II) compounds. It decomposes on heating to give PbO, NO_2 and O_2 and it is used as an oxidant in ignition mixtures.

Lead(II) sulphate, $PbSO_4$. This salt is precipitated from Pb(II) solutions by adding dilute sulphuric acid or a soluble sulphate. As the compound is practically insoluble in water it is used as a reagent for the detection of Pb(II) in gravimetric analysis. The compound is considerably more soluble in concentrated sulphuric acid, which suggests the formation of sulphate complexes. It also dissolves in concentrated alkali solutions.

Lead(II) chromate, $PbCrO_4$. This compound is found in nature as the mineral *crocoite* and is prepared by reacting solutions of lead(II) acetate and potassium dichromate in acid media.

Lead(II) acetate, $Pb(CH_3COO)_2$. This is a white crystalline solid that is prepared by dissolving lead(II) oxide or lead(II) carbonate in concentrated acetic acid. The compound is very soluble in water and its solutions are often used to prepare other compounds. A trihydrate, also called "sugar of lead" because of its sweet taste, can be prepared by dissolving lead(II) oxide in hot dilute acetic acid. The trihydrate is the most common commercial form of lead(II) acetate.

Some significant physical properties [48] for these lead(II) compounds are summarized in Table 8.

3.3.2.2. Pb(IV)

Only non-organometallic compounds will be discussed here. Pb(IV) compounds with Pb-C bonds will be described in Chapter 3.

Table 8
Some significant physical data for Pb(II) compounds

Name/Chemical formula	CAS number	Mol. mass	Density /g.cm^{-3}	Mp /°C	Solubility
Lead(II) oxide/ PbO (litharge)	1317-36-8	223.2	9.35	888	i H_2O,EtOH; s dil HNO_3
Lead(II) oxide/ PbO (massicot)	1317-36-8	223.2	9.64	stab>489	i H_2O,EtOH s dil HNO_3
Lead(II) sulphide/ PbS	1314-87-0	239.3	7.60	1118	i H_2O; s acid
Lead(II) selenide/ PbSe	12069-00-0	286.2	8.1	1078	i H_2O; s HNO_3
Lead(II) telluride/ PbTe	1314-91-6	334.8	8.164	924	i H_2O; i acid
Lead(II) fluoride/ PbF_2	7783-46-2	245.2	8.44	830	sl H_2O
Lead(II) chloride/ $PbCl_2$	7758-95-4	278.1	5.98	501	sl H_2O; s alk
Lead(II) bromide/ $PbBr_2$	100312-22-8	367.0	6.69	371	sl H_2O; i EtOH
Lead(II) iodide/ PbI_2	10191-63-0	461.0	6.16	410	sl H_2O; i EtOH
Lead(II) carbonate/ $PbCO_3$	598-63-0	267.2	6.6	dec ≈315	i H_2O
Lead(II) nitrate/ $Pb(NO_3)_2$	10099-74-8	331.2	4.53	470	vs H_2O; sl EtOH
Lead(II) sulphate/ $PbSO_4$	7446-14-2	303.3	6.29	1087	i H_2O, acid; sl alk
Lead(II) chromate/ $PbCrO_4$	7758-97-6	323.2	6.12	844	i H_2O; s alk,dil acid
Lead(II) acetate/ / $Pb(CH_3COO)_2$	301-04-2	325.3	3.25	280	vs H_2O

Abbreviations:. Acid, acid solutions; alk, alkaline solutions; dil, dilute; EtOH, ethanol
i, insoluble in; s, soluble in; sl, slightly soluble in; vs, very soluble in
(Adapted from Ref. 48)

Lead(IV) halides

The fluoride PbF_4 can be prepared as tetragonal and significantly stable needles by reacting PbF_2 with F_2 above 250 °C. The chloride $PbCl_4$ is prepared by passing chlorine gas into a suspension of $PbCl_2$ in hydrochloric acid at 0 °C, with the resulting chlorocomplex precipitated as ammonium hexachloroplumbate and then decomposed by adding it to cold sulphuric acid to render the free chloride.

Lead(IV) oxide, PbO_2. Present in nature as the somewhat rare mineral *plattnerite*, this compound can be prepared by the electrolytic or chemical oxidation of Pb(II) salts. Practically insoluble in water, it is somewhat soluble in acids and forms Pb(IV) salts. On heating above 550 °C this compound gives PbO and O_2. As a strong oxidizing agent it is used in the chemical industry and is an essential component of lead-acid batteries.

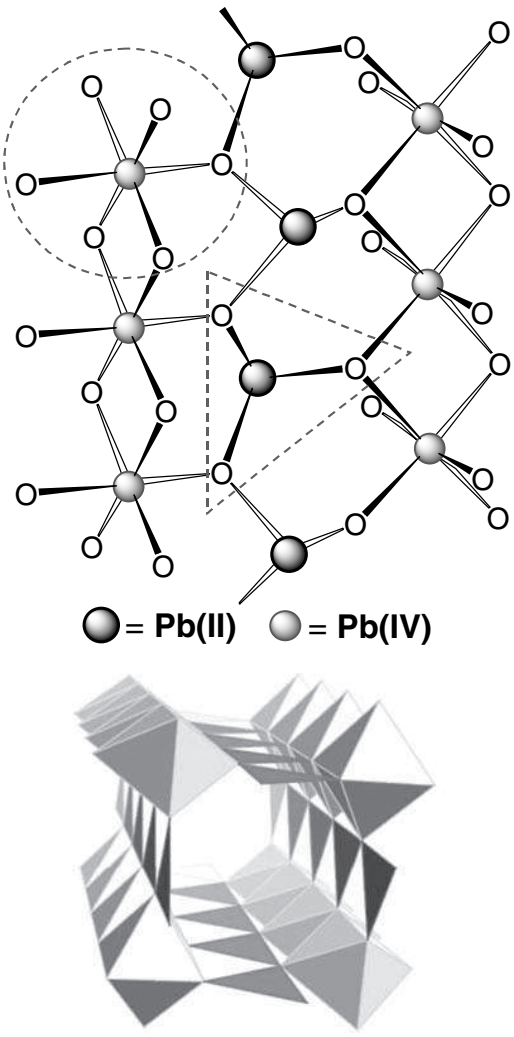


Figure 10. Structure of red lead Pb_3O_4 (minium) showing octahedra of $Pb^{IV}O_6$ and pyramids of $Pb^{II}O_3$.

Pb_3O_4, also called *minium* or red lead, is a brilliant orange-red pigment that is obtained by heating lead(II) oxide in air at 450-500 ºC. This compound decomposes above 550 °C to give PbO and O_2. This material contains Pb(IV) and Pb(II) in a structure that is composed of chains of Pb(IV)O_6 octahedra joined by their opposite edges; the chains are linked by Pb(II) ions coordinated in a pyramidal environment by three oxygen atoms. A fragment of the structure is shown in Fig. 10.

Table 9
Some significant physical data for Pb(IV) compounds

Name/Chemical formula	CAS number	Mol. mass	Density /g.cm^{-3}	Mp /°C	Solubility
Lead(IV) fluoride/ PbF_4	7783-59-7	283.2	6.7	≈ 600	
Lead(IV) chloride/ $PbCl_4$	13463-30-4	349.0		-15	
Lead(IV) oxide/ PbO_2	1309-60-0	239.2	9.64	290 dec	
Lead(II,II,IV) oxide/ Pb_3O_4	1314-41-6	685.6	8.92	830	i H_2O, EtOH; s hot HCl
Lead(IV) acetate/ / $Pb(CH_3COO)_4$	546-67-8	443.4	2.23	≈ 175	reac H_2O, EtOH; s bz, chl

Abbreviations: bz, benzene; chl, chloroform; EtOH, ethanol; i, insoluble in; reac, reacts with; s, soluble in.
(Adapted from Ref. 48)

The action of warm glacial acetic acid on Pb_3O_4 followed by cooling of the resulting solution gives *lead(IV) acetate*. This compound is a solid that is very sensitive to moisture and is widely used as an oxydizing agent in organic synthesis.

The significant physical properties [48] of these Pb(IV) compounds are summarized in Table 9.

4. APPLICATIONS

Lead is currently used for lead-acid storage batteries, for construction purposes (as sheets and pipes), for cable sheathing, radiation shielding, in alloys and other minor applications. Lead compounds are also present in batteries, PVC additives, pigments and other paint additives, glass, glazes and enamels and functional ceramics [6, 28].

4.1 Lead metal

The greatest use for lead today is in Lead-acid batteries (in 2004, 71% of the total world lead consumption was for this use [29]). Automotive batteries for starting, lighting and ignition (SLI batteries) are largely used. Furthermore, traction batteries are used to power electric vehicles and stationary batteries are used in systems and installations for which instant emergency back up power is required in case of power failure, such as computers, telecommunication systems and scientific instrumentation.

A storage battery or accumulator like this is a device that stores electrical energy as chemical potential energy. The electric energy in the "charge" step produces a chemical reaction that gives products that behave as an energy reservoir; this energy is then released as an electric current in the "discharge" step.

The simplest description of the battery is a tank of dilute sulphuric acid (2.4-4 M; density 1.15-1.22 $g.cm^{-3}$) into which two electrodes are introduced. The negative electrode is made of a grid of spongy metallic lead (antimony, calcium or tin are added to increase the strength, lifetime and resistance to fatigue) and the positive terminal is a grid filled with PbO_2. When the two electrodes are connected by an electric conductor the chemical reaction that occurs can be represented schematically as follows:

$$Pb + SO_4^{2-} \rightleftharpoons PbSO_4 + 2\,e^-$$

$$PbO_2 + 4\,H^+ + SO_4^{2-} + 2e^- \rightleftharpoons PbSO_4 + 2H_2O$$

$$PbO_2 + Pb + 2\,H_2SO_4 \underset{\text{charge}}{\overset{\text{discharge}}{\rightleftharpoons}} 2\,PbSO_4 + 2H_2O$$

As the discharge gets under way, the cell produces a voltage of 2V, sulphuric acid is consumed and both electrodes are covered by insoluble lead sulphate. To recharge the battery an external source of more than 2V must be applied in a reverse sense, converting the lead sulphate into lead and lead dioxide.

Even though these batteries are heavy, bulky and store only a limited amount of energy, they are currently the most economic way to store energy. Other alternatives, such as nickel/cadmium batteries, are useful only for specific applications or are still being tested.

Extruded lead is widely used; in fact, the manufacture of lead sheet is, after batteries, the second most important use of metallic lead. Most lead sheet is used in the building and construction industry, where typical applications are flashings and weatherings, complete roofing systems and vertical wall cladding. The characteristics of lead sheet, such as durability, low maintenance, recyclable nature and low energy requirement for conversion, still make this material useful in comparison to other, often cheaper, alternatives. However, although the demand in the UK is constant, probably due to the style of buildings, climatic conditions and its traditional usage, some of these alternatives based on aluminium, steel, zinc, copper or polymers are widely used in other countries.

To a lesser degree lead sheet is used for radiation shielding. The high density and atomic number of lead makes it useful to provide protection against gamma and X-ray radiation. This protection is necessary in medical installations and in the nuclear power industry.

The use of lead pipes for domestic water supplies, which has been a general application since Roman times, has reduced considerably; the replacement materials are copper or PVC. However, for specific industrial applications the corrosion resistant lead pipe is still used.

Lead is also used as a sheathing material for power cables in the petrochemical industry and undersea, as well as for high voltage underground cables. Lead is impervious to water, has good corrosion resistance, can be extruded in very long lengths, can be easily joined by soldering and can be applied to the cable core at moderate temperatures; despite these interesting characteristics, it is now being replaced in some applications by aluminium sheets or foil although replacement is still difficult for undersea cables.

Other examples of lead products include lead shot and other munitions, for which steel can be an alternative, or lead weights, with stainless steel or plastic coated iron as alternatives. Other historical uses include figures, ornaments and seals; in these cases lead has been replaced by other metals or plastic.

Lead metal in powder form is a component of paints, pigments and pastes when mixed with lead(II) oxide.

The use of lead in alloys constitutes another significant application because the addition of lead to other metals improves the properties of the resulting alloy. Tin-lead alloys were used as solders in ancient times (Table 1) and are still the most widely used today; their low melting points (the lowest melting point is 183 °C for a 38% lead/72% tin composition [28]), good flow characteristics and low price make them highly suitable for many applications particularly in the electronics industry, in which 10% of the lead consumed in the USA in 2000 was used [49].

Even though the USA government has not yet introduced regulations against the use of lead in electronics production, Japan required all new electronic products to be lead-free as of 2005 and the European Union will ban lead from these products in 2006 [49].

In response to this new legislation, lead-free alloys and electrically conductive adhesives are being tested as alternatives [49]. These alloys contain tin as a primary element plus silver, copper, bismuth, zinc, indium or nickel, with tin/silver/copper appearing to be the most promising. With respect to the adhesives, they still suffer from significant drawbacks for high-power devices due to conductivity fatigue in reliability tests, limited current carrying capability and poor impact strength.

Pewter and lead-tin alloys for ornaments and tableware are no longer manufactured in significant quantities. Lead-tin alloys and bronzes with a content of up to 20% lead are still used for bearings and small amounts of lead are added to modern copper, aluminium, bronze and steel alloys to improve machinability.

4.2. Lead compounds

The addition of lead(II) oxide to glass has been known since Antiquity and was discussed above. This addition not only causes a dramatic reduction in the melting temperature of glass, making it softer and easier to cut, but also increases the refractive index, making the glass not only more attractive but giving it excellent optical properties. These properties together with its high density make leaded glass useful for radiation shielding, in applications ranging from the manufacture of cathode ray tubes or fluorescent tubes to electric glass. Leaded glass is also used to protect people from the danger of radiation from X-ray equipment or radioactive materials in hospitals, laboratories and nuclear power stations.

The incorporation of lead compounds into PVC gives good stability to heat and UV light, good electrical and mechanical properties and good processing behaviour. These reasons make lead salts, for example lead(II) carbonate and a basic sulphate of composition $3PbO.PbSO_4.H_2O$, the most cost-effective stabilizers and these are nowadays widely used for rigid PVC products.

The ancient use of lead compounds in glazes and enamels is in steady decline, although for specific products litharge is still used. In addition, some compounds such as lead(II) cyanamide, lead(II) oxide (litharge), the basic lead(II) carbonate $2PbCO_3.Pb(OH)_2$, Pb_3O_4 and lead(II) chromate are still used for specific paints and pigments. In particular, Pb_3O_4 (or minium) is widely used as a protective agent to prevent iron or steel from rusting in a moist atmosphere.

As a photoconductor, lead(II) sulphide is used in photographic light-meters and photoelectric cells and as a radio receptor it was widely used in early crystal radio receivers.

Ceramics containing lead(II) zirconate or lead(II) titanate have piezoelectric properties and have a limited use.

Apart from this wide range of uses [6, 28], several of the lead compounds mentioned above, as well as other lead(II) and lead(IV) compounds, have specific applications in laboratories or in industry. A detailed overview of these applications can be found in references 27 and 50.

Acknowledgment. Authors thank Dr. María C. Touza and María Sordo for their help with the figures.

REFERENCES

[1] J. O. Nriagu, Lead and Lead poisoning in antiquity, John Wiley, New York, 1982.
[2] D. Seiferth, Organometallics, 22 (2003) 2346, 5154.
[3] P. Grandjean and T. Nielsen, Residue Rev., 72 (1979) 97
[4] J. O. Nriagu, Sci. Tot. Environm., 31 (1983) 105.
[5] D. M. Settle and C. C. Patterson, Science, 207 (1980) 1167.
[6] Lead development association international. http://www.ldaint.org

[7] R. J. Hopper, Ann. British School Athens, 63 (1968) 293.
[8] R. Gea Javaloy, Ind. Min., 28 (1986) 11.
[9] M. B. Abbott and A. P. Wolfe, Science, 301 (2003) 1893.
[10] C. Giardino, Il metalli nel mondo antico, 2nd Ed., Laterza, Roma, (1999)
[11] M. Murozumi, T. J. Chow and C. Patterson, Geochim. Cosmochim. Acta, 33 (1969) 1247.
[12] J. O. Nriagu, Science, 272 (1996) 223.
[13] S. Hong, J.P. Candelone, C. C. Patterson and C. F. Boutron, Science, 265 (1994) 1841.
[14] I. Renberg, M. W. Persson and O. Emteryd, Nature, 368 (1994) 323.
[15] M. L. Brännvall, R. Bindler, I. Renberg, O. Emteryd, J. Bartnicki and K. Billström, Environm. Sci. Technol., 33 (1999) 4391.
[16] A. Martinez Cortizas, E. García-Rodeja, X. Pontevedra Pombal, J. C. Nóvoa Muñoz, D. Weiss and A. Cheburkin, Sci. Tot. Environm., 292 (2002) 33.
[17] E. S. Claudio, H. A. Godwin and J.S. Magyar, Progress Inorg. Chem., 51 (2003) 1.
[18] J. O. Nriagu, New Eng. J. Med., 308 (1983) 660.
[19] J. Eisinger, Nat. Hist., 105 (1996) 48.
[20] S. C. Gilfillan, J. Occup. Med., 7 (1965) 53.
[21] S. Hernberg, Am. J. Ind. Med., 38 (2000) 244.
[22] J. A. Witkowski and L. C. Parish, Clin. Dermatol., 19 (2001) 367.
[23] H. W. Mielke, Am. Sci., 87 (1999) 62.
[24] H. L. Needleman, Environm. Res., 74 (1997) 95.
[25] H. L. Needleman, Environm. Res., 78 (1998) 79.
[26] C. Patterson, J. Ericson, M. Manea-Krichten and H. Shirahata, Sci. Tot. Environm., 107 (1991) 205.
[27] F. Habashi (ed.), Handbook of Extractive Metallurgy, Vol II. VCH, Weinheim, 1997.
[28] I. Thornton, R. Rautiu and S. Brush, Lead the facts, Ian Allan Printing, Surrey, 2001.
[29] International lead and zinc study group. http://www.ilzsg.org
[30] M. J. Scoullos (ed.), Mercury-Cadmium-Lead Handbook for Sustainable Heavy Metals Policy and Regulation, Kluwer, Dordrecht, 2001.
[31] N. N. Greenwood and A. Earnshaw, Chemistry of the Elements, 2nd Ed, Butterworth-Heinemann, Oxford, 1997.
[32] J. Emsley, The Elements, 2nd Ed, Oxford University Press, Oxford, 1998.
[33] B. Wrackemeyer, Ann. Rep. NMR Spect., 47 (2002) 1
[34] E. S. Claudio, M. A. tert Host, C. E. Forde, C. L. Stern, M. K. Zart and H. A. Godwin, Inorg. Chem., 39 (2000) 1391.
[35] J. S. Casas, E. E. Castellano, J. Ellena, M. S. García Tasende, A. Sánchez, J. Sordo and M. J. Vidarte, Inorg. Chem., 42 (2003) 2584.
[36] L. A. Margolis, C. D. Schaeffer Jr and C. H. Yoder, App. Organomet. Chem., 17 (2003) 236.
[37] A. M. Sawvel, S. C. Chinn, W. L. Bourcier and R. S. Maxwell, Chem. Mat., 17 (2005) 1493.
[38] N. V. Sidgwick, The Chemical Elements and their Compounds, Clarendon Press, Oxford, 1950.
[39] P. Schwerdtfeger, G. A. Heath, M. Dolg and M. A. Bennett, J. Am. Chem. Soc., 114 (1992) 7518.
[40] M. Kaupp and P. v. R. Schleyer, J. Am. Chem. Soc., 115 (1993) 1061.
[41] T. S. Hofer and B. M. Rode, J. Chem. Phys., 121 (2004) 6406.
[42] S. Kobayashi, S. Nagayama and T. Busujima, J. Am. Chem. Soc., 120 (1998) 8287.
[43] W. Nimal Pereira, G. Hefter and P. M. Sipos, Inorg. Chem., 40 (2001) 3974.

[44] S. M. Grimes, S. R. Johnston and I. Abrahams, J. Chem. Soc., Dalton Trans., (1995) 2081.
[45] T. G. Spiro, D. H. Templeton and A. Zalkin, Inorg. Chem., 8 (1969) 856
[46] A. F. Holleman and E.Wiberg, Inorganic Chemistry, Academic Press, San Diego, 2001.
[47] A. Walsh and G. W. Watson, J. Solid State Chem., 178 (2005) 1422.
[48] D. R. Lide (ed.), Handbook of Chemistry and Physics, 80th Ed, CRC Press, Boca Ratón, 1999.
[49] Y. Li, K-S- Moon and C. P. Wong, Science, 308 (2005) 1419.
[50] R. E. Kirk and D. F. Othmer (eds.), Kirk-Othmer. Encyclopedia of Chemical Technology. Vol. 15. 4th Ed, John Wiley&Sons, New York, 1991.

Lead - J.S. Casas and J. Sordo (Editors)

Chapter 2

Lead(II) coordination chemistry in the solid state

J. Sergio Casas, José Sordo and María J. Vidarte

Department of Inorganic Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

1. INTRODUCTION

Most of the inorganic chemistry of lead is associated with the (II) oxidation state. The few compounds containing Pb(IV) that have been well characterized are all strong oxidizing agents. The opposite is true in organolead chemistry. This rather striking division has been traditionally associated with the "inert-pair effect" [1] and, more recently, to the differences in the radial extension of s- and p-orbitals when electronegative substituents are bound to the metal. If these differences increase, then the sp^n hybridization becomes more unfavourable, the Pb–X bonds become weaker and the (IV) oxidation state becomes more unstable [2].

This chapter deals with the coordination chemistry of Pb(II) in the solid state, a particularly fascinating field that has experienced a clear resurgence in the last few decades. The numerous applications of the metal in technology (see Chapter 1), its widespread occurrence in the environment and public concern about its toxic effects (see Chapter 3) have markedly increased the attention paid to its interaction with both natural and non-natural ligands.

It must be noted that Pb(II) is a rather large ion (see Table 1) and behaves as a borderline acid according to the HSAB principle of Pearson [3]. These properties permit a broad range of coordination numbers (CN) in its complexes and make the selection of the donor atoms flexible. In accordance with this, Pb(II) complexes exhibit CNs from quasi-one to twelve, while O, N, S, P, Cl, Br or I donor atoms are common in its coordination sphere.

There are some additional problems that make lead coordination chemistry more complicated. In many cases the distances between the lead and the donor atoms of its ligands are widely spread, making it very difficult to establish the exact coordination number of the metal. Although the sum of the van der Waals radii is normally a good criterion to fix an upper limit for the distances belonging to secondary metal-to-ligand interactions, the more widely

accepted value for r_{vdW} in the case of Pb(II) (2.00 Å, Table 1) may be underestimated [4].

Table 1.
Radii of Pb(II) (Å)

Ionic radius	Cov. radius	v. d. W. radius
1.32[a]	1.54[a]	2.00[b]

[a]J. Emsley, *The Elements*, Oxford Univ. Press, N.Y., 1998
[b]J.E. Huheey, E.A. Keiter and R.L. Keiter, Inorganic Chemistry, Fourth Edition, Harper Collins, 1993.

The use of the aforementioned criterion therefore does not always contribute to define the coordination number in lead complexes. In such situations, some authors accept as significant contacts those that, although longer than the "normal" bond distances, are much shorter than the next most distant contact.

Another aspect is that Pb(II) stereochemistry is conditioned by the structural effect of the $6s^2$ electron pair. Whether or not this electron pair is "stereochemically active" has a very significant influence on the geometry of Pb(II) coordination compounds (*vide infra*).

All of these factors contribute to a particularly fascinating coordination chemistry with relevance in many areas of knowledge, extending from theoretical chemistry to the fields of ecology and toxicology.

This Chapter is based on the reviews published by Harrison [5, 6], Parr [7, 8], Holloway and Melnik [9], Glusker et al. [10] and Godwin et al. [11]. All of this information was updated using a structural search of the Cambridge Structural Database (CSD) [12] and a SciFinder® Scholar survey of the recent literature.

Readers interested in the coordination chemistry of lead(II) in solution – namely the kinetics and thermodynamics of the lead/ligand interactions – are referred to the work of Godwin et al. [11] for a recent discussion of this topic.

2. THE STRUCTURAL RELEVANCE OF THE LEAD(II) $6s^2$ LONE PAIR

Pb(II) has an electronic structure $[Xe]4f^{14}5d^{10}6s^2$. Due to relativistic effects, which are maximum at Au(I) but also operative in other close 6p metals such as lead, the 6s orbital is contracted and stabilized. As mentioned above, this stabilized 6s pair reduces its participation in the chemistry of the element (becoming an "inert-pair") and this explains why inorganic Pb forms compounds in a lower oxidation state (less by two) than would be expected from its group number (see 3.3.2 in Chapter 1).

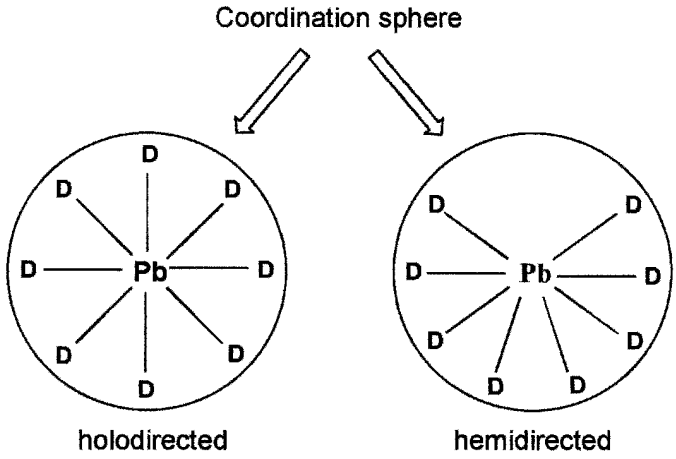


Figure 1. Stereochemical influence of the 6s pair (D = donor atom of a ligand) (Adapted from Ref. 10).

The apparent reticence of the 6s electrons to play a role in the chemistry of the element may also affect the stereochemistry of Pb(II) complexes. This influence can be understood in terms of simple hybridization or valence shell electron-pair repulsion arguments (an alternative approach to this topic has recently been published [13]). On using the former approach, it seems that the 6s orbital, in spite of its stabilization, can hybridize with the 6p orbitals to give a "stereochemically active" 6s electron pair (or stereochemically active lone electron pair, *SALEP*) occupying one position in the coordination sphere of the metal. Because the pair is not directly detectable, its presence is normally identified by a void in the distribution of the coordination bonds (*hemidirected* coordination, see Fig. 1).

If hybridization does not occur and the pair has only s character, then it is "stereochemically inactive" and the complex does not show a gap or void in the bond distribution (*holodirected* coordination, see Fig. 1) [10].

In this respect, some regularity was observed in the CSD search carried out by Glusker et al. [10]. For low CN values (from 2 to 5) all Pb(II) compounds show hemidirected geometries. One recent example is the derivative of the tetrakis(imidazolyl)borate anion $[Pb\{B(Im)_4\}]NO_3(xH_2O)$ (x = 0–1.5) prepared by reacting an H_2O/EtOH solution of $Na\{B(Im)_4\}$ and aqueous $Pb(NO_3)_2$ [14].

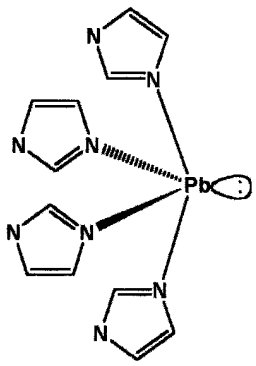


Figure 2. Hemidirected centre in $[Pb\{B(Im)_4\}]NO_3$ (Adapted from Ref. 14).

In this complex each Pb(II) atom is coordinated by four imidazole rings from different borates, giving rise to a hemidirected centre (see Fig. 2).

It was also concluded that structures with coordination numbers 9 and 10 are all holodirected, although it was pointed out that relatively few structures with these high coordination numbers were included in CSD when the database was reviewed [10]. Finally, for intermediate coordination numbers (6–8), both hemidirected and holodirected coordination geometries occur [10].

There are several factors that can "favor (but not rigorously determine)" [10] the adoption of a hemi- or holodirected structure by a complex. These factors can be summarized with the following "rules": *(i) low coordination numbers, hard ligands (O or/and N donor atoms) and attractive interactions between them* (e.g. chelate or multidentate ligands) *all favour a hemidirected stereochemistry; (ii) high coordination numbers, soft ligands* (e.g. Cl^-, Br^- and I^-) *and repulsive interactions between them lead to holodirected structures* [10]. Although these two "rules" are not infallible, they can be useful in the aprioristic design of lead(II) complexes with specific structural characteristics.

A very good illustration of the relationship between the low CNs with the hemidirected situation and the higher CNs with the holodirected one is provided by the apatite-related compound $Pb_5(SiO_4)(VO_4)_2$ [15]. The structure of this compound has two non-equivalent Pb(II) centres, as shown in Fig. 3. Pb(1) is coordinated to six O ligands, which are positioned at the vertices of a pentagonal pyramid. The void in the coordination sphere of the metal is clearly observable and strongly suggests a SALEP. The Pb(2) metal centre, in turn, has a CN 9 with a distorted coordination but, nevertheless, without an obvious gap in the coordination sphere. So Pb(1) has a hemidirected coordination and Pb(2) a holodirected one.

However, the situation is not always as clear as this. In fact, the association of higher CNs with holodirected structures does have some exceptions.

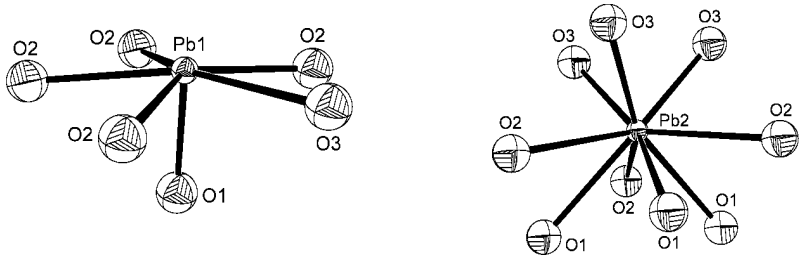


Figure 3. The two non-equivalent Pb(II) centres in $Pb_5(SiO_4)(VO_4)_2$ [15].

For example, the complex $[Pb(BzImH)_2py(H_2O)_2(NO_3)_2]\cdot(BzImH)_2py\cdot\cdot H_2O$, prepared by reacting aqueous lead(II) nitrate with an alcoholic solution of the 2,6-bis(2-benzimidazolyl)pyridine ligand, contains the metal centre coordinated to the three nitrogen atoms of the organic ligand, to the four oxygen atoms of the nitrate anions and to the two oxygen atoms of the water molecules, resulting in a nine-coordinated lead(II) structure. In spite of this high coordination number, the arrangement of the donor atoms suggests the presence of a gap, as occurs when the 6s pair is stereochemically active [16]. Several additional examples will be discussed in Section 3.5.

In case of doubt, it can be useful to use some complementary rules to ascertain the presence of a SALEP [17]. It seems that the presence of such a pair leads to: (i) a shortening of the Pb–D bond(s) (see Fig. 1) opposite to the hypothetical site occupied by the lone pair, a situation that leads to unusually short distances for a bond of that type, and (ii) a progressive lengthening of the other Pb–D bonds in the hemidirected stereochemistry as one moves toward the lone pair site.

The application of these rules also supports the proposed hemidirected coordination in the complex $[Pb(BzImH)_2py(H_2O)_2(NO_3)_2]\cdot(BzImH)_2py\cdot H_2O$. In this compound, the Pb–O bond distance (2.542 Å) associated with the water molecule opposite to the putative stereochemically active lone pair is clearly shorter than the Pb–O bond distance to the water molecule located near the gap (2.859 Å) [16].

A more elaborate way to assess the structural distortion induced by the 6s electron pair is to plot the Pb–D bond length against the lone-pair–Pb–D angle [17]. The location of the lone pair in a structure can be determined as follows: (i) if there is a rotational axis of symmetry, the pair should lie on this axis, which will be the only symmetry axis, and a gap in the coordination sphere of the metal or very long Pb–D bonds should be observable at the position of the lone pair; (ii) if there is no axis of symmetry in the complex, the lone pair can be located by looking for unusually short Pb–D bonds that, as mentioned above, should be located opposite the pair. When there are two short bonds, then the axis passing

through the lone pair should bisect the angle between these bonds. In complexes where three short bonds are present, the axis through the lone pair should pass through the centre of the triangle defined by the three bonds, etc.

As shown in Fig. 4, the plot associated with a holodirected structure is a line parallel to the abscissa axis, which means that there is no variation in the Pb–D bond lengths when one moves around the coordination sphere. The structures with increasing hemidirected distortion produce curves that progressively separate from the holodirected line [17].

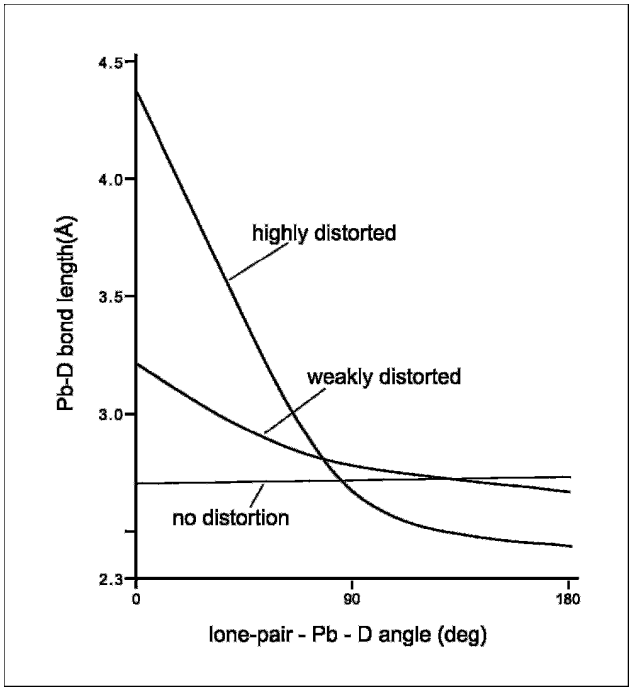


Figure 4. Plot of Pb–D bond length versus lone-pair–Pb–D angle for holodirected and hemidirected complexes (Adapted from Ref. 17).

Even with the aid of these complementary rules, sometimes it is not a straightforward task to classify a structure as hemidirected or holodirected due to the problems in establishing unambiguously the exact identity of the lead coordination sphere. A good example of this type of situation is found when the structure of the lead(II) carboxyethylphosphonate $[Pb_3(O_2CCH_2CH_2PO_3)_2]$ is analyzed. This complex has a structure consisting of three crystallographically independent Pb(II) centres with different coordination numbers that were formerly defined as 3, 4 and 5. At the same time each centre has a SALEP,

which occupies the fourth, fifth and sixth coordination positions, respectively [18]. Such hemidirected coordination spheres were apparently established by assuming an upper limit for the Pb–O bonds of 2.75 Å. However, if the cut-off distance that defines such bonds is increased to 2.9 Å, then the coordination numbers of the Pb(II) centres become 5, 6 and 7 [19a] and Pb(3) probably reaches a holodirected coordination (see Fig. 5). It is worth noting that in these examples both limits for Pb–O bond lengths are longer than the sum of the covalent radii (2.28 Å) but clearly shorter than the sum of the van der Waals radii (3.50 Å) (see Table 1 and references therein). This suggests that even the longest bonds close to 2.9 Å can be considered as significant bonding interactions. Another interesting example has recently been discussed by Harrowfield [19b].

Analysis of Pb–O bond distances in the CSD points to the same conclusion because there are many examples of structures containing Pb–O bond lengths equal to or greater than 2.9 (see Fig. 6), despite the fact that the mean value for this distance is 2.613 Å and the number of structures with bond distances longer than 2.7 included in the database diminishes quickly.

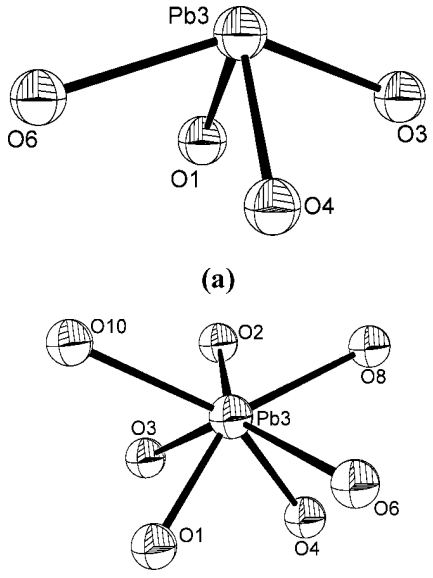


Figure 5. Coordination geometry for the Pb(3) centre in the $[Pb_3(O_2CCH_2CH_2PO_3)_2]$ complex (CSD Ref. DINDUZ, [18]) when an upper limit of 2.75 **(a)** or 2.9 Å (**b**) for the Pb–O bond distance is considered.

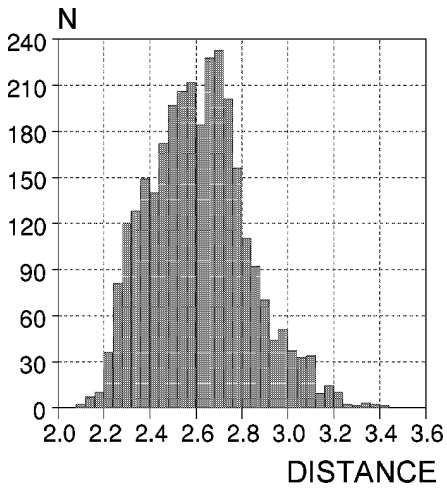


Figure 6. Histogram showing the number of bonds (N) versus the Pb–O bond distance (DISTANCE) for the complexes included in the CSD [12]. The minimum, maximum and mean values in the X-axis are 2.100, 3.413 and 2.613 Å, respectively.

The appropriateness of a careful analysis of all the short contacts between the Pb(II) centre and the surrounding donor atoms, even including the intermolecular interactions, before establishing whether the coordination is holo- or hemidirected, is clearly illustrated by the complex $[Pb(DBM)_2]$ (HDBM = 1,3-diphenylpropane-1,3-dione) [20]. At first sight, the lattice of this compound comprises centrosymmetric dimeric units $Pb_2(DBM)_4$ with two apparently hemidirected $\{PbO_5\}$ kernels. However, a closer look at the structure indicates the possible presence of a η^2 Pb···C interaction with a phenyl group for each metallic centre. Furthermore, there is long-standing evidence that lead can interact with phenyl rings to give hexahapto (η^6) contacts [see, e.g., Ref. 21]. In the present case, if the intermolecular approach between dimeric units is considered, then a η^6 Pb···C interaction between each Pb(II) and a phenyl group from a neighbouring unit also becomes apparent. All of these contacts lead to a "new" irregular but holodirected $\{PbO_5C_8\}$ kernel [20].

The last intermolecular hexahapto interaction brings to the fore an interesting issue. Can Pb(II) behave as a Lewis base, as Sn(II) does, through the 6s lone pair? Can the hexahapto interaction be a bond involving this lone pair, with the aromatic ring depleted in electron density acting as the acceptor? Unfortunately, this question does not have an easy answer because the opposite interpretation, with the metal behaving as a Lewis acid, is also possible.

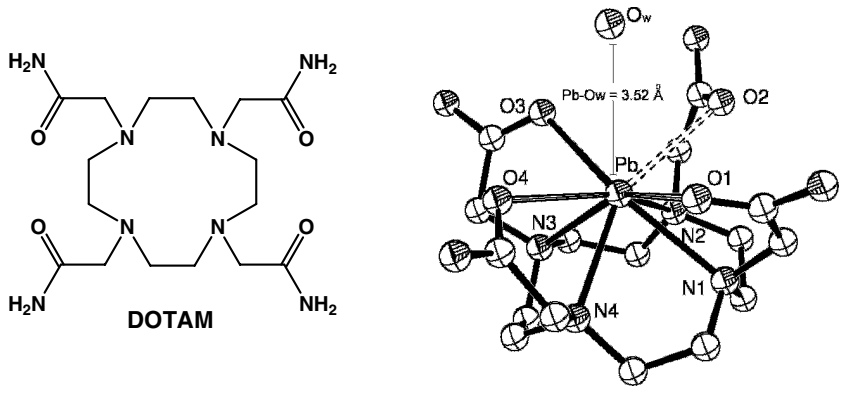


Figure 7. (a) The ligand DOTAM and (b) the structure of one of the two cations $[Pb(DOTAM)]^{2+}$ (CSD Ref. AYOSIQ, [17]) and the orientation of the water molecule.

This issue – albeit in a different context – has been addressed recently by Hancock et al. [17]. They prepared the complex $[Pb(DOTAM)](ClO_4)_2 \cdot 4.5H_2O$, where DOTAM is a tretraaza macrocycle with amide pendant arms (see Fig. 7).

According to an X-ray diffraction study, the complex contains two distinct $[Pb(DOTAM)]^{2+}$ cations, both with a hemidirected structure and the metal coordinated to four N-donors from the macrocycle and four O-donors from the pendant arms. These cations differ in the identity of the atoms located a long distance above the gap in the coordination sphere: a water molecule in one case and the amide nitrogen in the other. In the former case, the oxygen atom of the water molecule is 3.52 Å above the putative site of the lone pair (see Fig. 7).

Although locating H atoms near a heavy Pb atom in an X-ray study is not an easy task, careful analysis of the data led to the conclusion that one of the hydrogens of the water molecule is close to the surface of the Pb(II) ion, possibly giving rise to a hydrogen bond that involves the stereochemically active lone pair (i.e., a Pb–lone-pair···H–O hydrogen bond). Nevertheless, it was concluded [17] that there is no H-bond, but that the presence of the lone pair allows a closer approach between the hydrogen and the metal centre than would be possible in the absence of the lone pair.

A final, unsuspected role for the 6s lone pair has recently been proposed. It is related with the toxic effects of lead compounds, a problem that will be discussed in detail in Chapter 4. Briefly, lead poisoning causes anaemia because it interferes with heme synthesis; heme is the prosthetic group of haemoglobin and other biomolecules. This interference in the heme biosynthesis pathway is related to the capacity of lead to behave as an inhibitor of the coproporphyrinogen oxidase, ferrochelatase and 5-aminolaevulinic acid dehydratase (ALAD) enzymes, with the effect being more marked on the latter

system (see Chapter 4, 5.2.1). ALAD (which is also known as porphobilinogen synthase) in animals, fungi, archaea and some bacteria contains an essential catalytic site with zinc [22]. This site has a rather unusual amino acid composition for a catalytic zinc, namely three cysteine ligands. The metal is essential for binding one of the substrate molecules (δ-aminolevulinic acid) through the carbonyl oxygen and the amino nitrogen atoms [22].

In the presence of lead, the zinc is displaced from ALAD and the catalytic centre is occupied by the lead. Although the lead maintains the three bonds with the cysteine ligands (see Fig. 8), unlike the native metal, it has a SALEP that tempers the electrophilicity of the catalytic centre, eventually leading to enzyme inhibition [23].

This may not be an isolated example of the differences between lead(II) and zinc(II) coordination behaviour in biological systems. As pointed out recently [24], in cases of lead poisoning the preference of lead(II) for Pb(II)-S_3 coordination instead of Pb(II)-S_4 could also be responsible for the improper folding of the transcription factors that contain cysteine-rich (Cys_4) zinc-binding domains, thus reducing or preventing their binding to DNA. This phenomenon could be one of the molecular mechanisms that contributes to the toxic effects of lead(II).

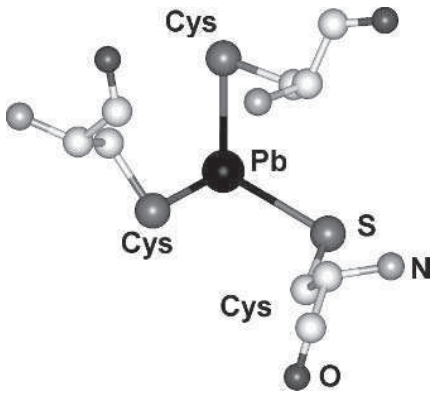


Figure 8. $Pb(Cys)_3$ centre in yeast 4-aminolaevulinic acid dehydratase lead complex (1QNV, Protein Data Bank).

3. STRUCTURAL CHARACTERISTICS OF THE COORDINATION COMPOUNDS OF LEAD(II) IN THE SOLID STATE: SOME RELEVANT EXAMPLES

In this section, the more relevant aspects of the coordination of lead(II) in the solid state will be discussed. This discussion is not comprehensive, comprising only some interesting examples that are ordered by increasing coordination numbers of the metal. The reader may refer to more specialized works for more complete coverage of this rather large field [5-9, 11]. The examples selected throughout this section will exclude compounds with C–Pb bonds because these systems are considered at length in Chapter 3. Only occasionally is one of these compounds included and this is due to its particular relevance to the matter under discussion. If the specific structure of a complex is alluded to, then the corresponding figure caption will include its CSD reference, as in the previous section.

3.1. Coordination numbers lower than four

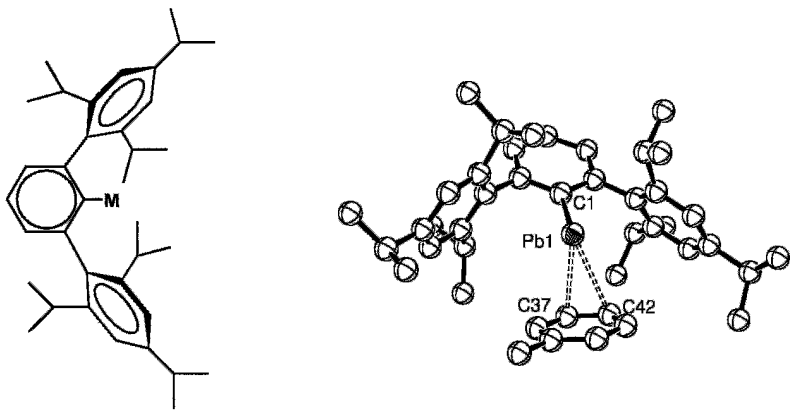
Examples of "inorganic" lead(II) complexes with a coordination number lower than two are not known. The only compound with *quasi-one-coordinate Pb(II)* is an organometallic derivative containing a strong C-Pb(II) bond as well as a weak interaction with a solvent molecule (Fig. 9). This interaction is with a toluene molecule but only involves two of the carbon atoms from the toluene ring and, as such, can be described as being of the η^2 type [25].

The compound was prepared using the steric influence of the bulky monodentate terphenyl ligands. This type of ligand (see Fig. 9a) wraps around the metal centre and hinders the approach of additional coordination entities – a situation that leads to very low coordination numbers and some interesting chemistry [26].

The synthesis of the quasi-one-coordinate lead complex was achieved by reacting (2,6-$Trip_2$-C_6H_3)-Pb-Me with one equivalent of $B(C_6F_5)_3$ in toluene:

$$(2,6\text{-}Trip_2\text{-}C_6H_3)\text{-}Pb\text{-}Me + B(C_6F_5)_3 \xrightarrow{\text{toluene}}$$

$$[Pb(2,6\text{-}Trip_2\text{-}C_6H_3)(\text{toluene})][B(C_6F_5)_3Me] \qquad (1)$$

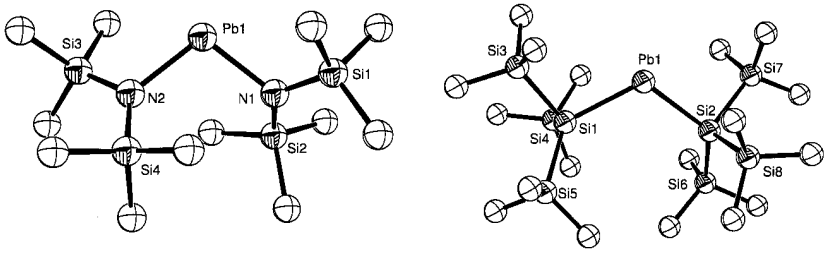


(a) **(b)**

Fig. 9. (a) The bulky monodentate ligand (2,6-$Trip_2$-C_6H_3) bound to a metal M showing the steric impediment; (b) structure of the [Pb(2,6-$Trip_2$-C_6H_3)(toluene)]$^+$ cation of the [Pb(2,6-$Trip_2$-C_6H_3)(toluene)][$B(C_6F_5)_3Me$] complex (BEWKAQ, [25]) showing the η^2-bound toluene molecule at the bottom.

The terphenyl ligand is bound to the metal centre through a strong C(1)–Pb bond [2.250(7) Å] (Fig. 9b), which is marginally shorter than the same bond in (2,6-$Trip_2$-C_6H_3)-Pb-Me [2.272(9) Å]. Additionally, the lead(II) interacts weakly with a molecule of toluene that is also included in the lattice. Analysis of the distances between the metal and the toluene ring suggests that the weak interaction is of the η^2 Pb··C type and involves the C(37) and C(42) carbon atoms, which are located at distances of 2.832(10) and 2.907(9) Å from the lead(II), respectively. Thus, the coordination number of the metal centre is not strictly one. Nevertheless, this very low coordination number is due not only to the bulkiness of the ligand but also to the low basicity of the tetraorganoborate counterion [see Eq. (1)]. In fact, the same ligand does allow the approach of some other bulky groups to the lead atom, as will be seen below.

A review of the literature during the period 1995–1996 was carried out by Holloway and Melnik [9] and includes eleven examples of lead(II) compounds with the *coordination number two*. Of these eleven, four are organometallic compounds, several also include additional metal-to-ligand secondary interactions and some do have such interactions although they are not specifically mentioned by the authors. A survey of the Cambridge Structural Database, focussed on the structures published between 1996 and 2004, detected two more examples, one of which contains Pb–M bonds.



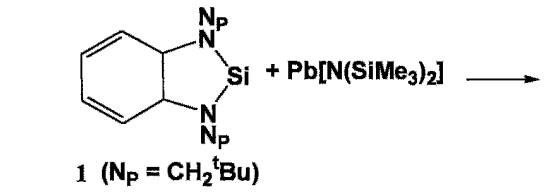
(a) **(b)**

Figure 10. Structure of (a) $[Pb\{N(SiMe_3)_2\}_2]$ (BUGRUQ, [27]) and (b) $[Pb\{Si(SiMe_3)_3\}_2]$ (ZAHTIL, [28]). The hydrogen atoms have been omitted for clarity.

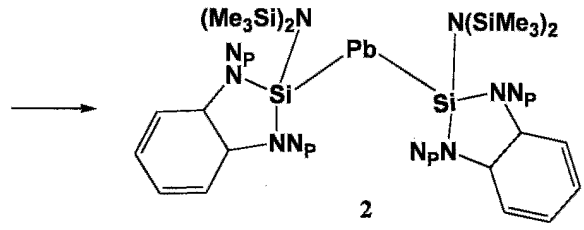
As occurs with the coordination number quasi-one, genuine lead(II) compounds with a coordination number two arise when very bulky ligands are present and in these cases the stereochemistry is always angular – with the only exception containing Pb–M bonds. The first example is the stable lead(II) bis(trimethylsilyl)amide $[Pb\{N(SiMe_3)_2\}_2]$, which was studied by X-ray diffraction in the solid state (Fig. 10a) and by electron diffraction in the gas-phase [27].

The average value of the Pb–N distance is slightly longer in the crystal [2.24(2) Å] than in the gas-phase [2.20(2) Å] but the difference in the N–Pb–N angle is bigger [103.6(7)° and 91(2)°, respectively]. Although the solid state angle is consistent with sp^2 hybridization for the metal atom with a SALEP at one of the hybrid orbitals, the angle in the gas-phase would be compatible with the absence of hybridisation and the involvement of pure 6p orbitals in the Pb–N bonds. However, Fjeldberg et al. [27] relate the difference in the angles to the fact that packing the molecules in the lattice gives rise to close intramolecular contacts, meaning that the NPbN angle opens and ensures the relief of steric strain.

This compound is a good starting point for the preparation of other complexes with two-coordinated lead(II). For example, reaction of the complex with the [tris(trimethylsilyl)silyl] derivative of a heavy alkali metal in *n*-pentane at –60 °C affords $[Pb\{Si(SiMe_3)_3\}_2]$ (Fig. 10b) [28]. Reaction with silylene **1** gives the bis(silyl)lead(II) compound **2** [29]:



1 (N_P = $CH_2{}^tBu$)

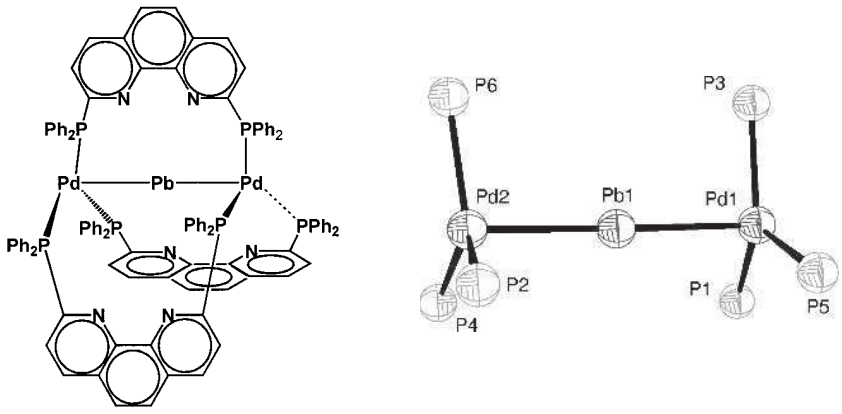


2 (2)

Both $[Pb\{Si(SiMe_3)_3\}_2]$ and **2** are angular complexes, with Si–Pb–Si bond angles of 113.56(10)° and 105.8(1)°, respectively, and their structures are therefore compatible with the presence of a SALEP. In accordance with this situation, a reaction pathway involving an intermediate was suggested for the process in Eq. (2), in which $[Pb\{N(SiMe_3)_2\}_2]$ behaves both as a Lewis base and as a Lewis acid [29].

A very different situation has been observed in the complex $[Pd_2(P_2phen)_3Pb](ClO_4)_2$, where P_2phen is 2,9-bis(diphenylphosphino)-1,10-phenanthroline [30]. Each of the P_2phen bismonodentate ligands in the structure bridges the two Pd(0) centres, which thus become three-coordinated. These two trigonal centres behave as if they are metallophilic, each forming a bond with the lead(II) ion (Fig. 11a). The "box-like" $Pd_2(P_2phen)_3$ moiety formally behaves as a host while Pb(II) is the guest ion.

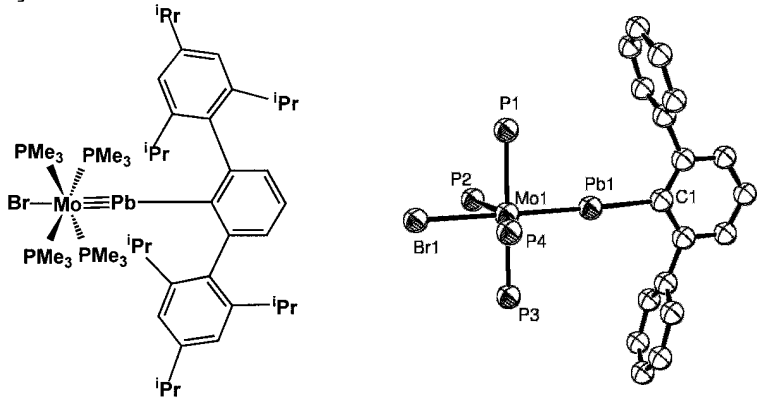
The Pb–Pd bond distances are almost identical [2.7095(6) and 2.6902(6) Å] and the kernel Pd–Pb–Pd (Fig. 11b) is practically linear [178.75(1)°], indicating a stereochemically inactive 6s lone pair on the lead. In fact, Catalano et al. [30] rationalized the metal-metal interaction using a qualitative MO diagram that combines the 6s lead orbital with the $4d_z^2$ palladium orbital, both of which are filled, and the empty $6p_z$ lead orbital with the empty $5p_z$ palladium orbital. The result of this combination is a stabilization of the filled orbitals relative to the unfilled ones, a situation that leads to a bonding interaction between the three metals. So, in this complex and according to this proposal, the 6s pair is involved in the bond. An earlier Pt–Pb complex showed the same type of metallic arrangement (a linear Pt–Pb–Pt fragment) but it also contained eight relatively strong F···Pb interactions [31].



(a) (b)

Fig. 11. (a) General arrangement of the cation $[Pd_2(P_2phen)_3Pb]^{2+}$ (QAZGIH, [30]) and (b) the coordination sphere of the two types of metal in the cation.

Interestingly, the quasi-one-coordinate lead(II) complex cation $[Pb(2,6\text{-}Trip_2\text{-}C_6H_3)]^+$ (see Fig. 9a), despite the constraint produced by the bulky terphenyl ligand, is able to form a Pb-Mo triple bond when $[Pb(2,6\text{-}Trip_2\text{-}C_6H_3)Br]_2$ reacts with the dinitrogen complex *cis*-$[Mo(N_2)_2(PMe_3)_4]$ in toluene at room temperature [32a]. Similar reactions occur when $[Pb(2,6\text{-}Trip_2\text{-}C_6H_3)X]_2$ (X = Br, I) complexes interact with $[W(N_2)_2(PMe_3)_4]$ in the same solvent at 100 °C [32b].



(a) (b)

Figure 12. (a) Scheme of the $[Br(PMe_3)_4Mo{\equiv}Pb(2,6\text{-}Trip_2\text{-}C_6H_3)]$ complex and (b) view of the Pb(II) centre and the surrounding atoms in the complex (BEQYEC, [32a]). The methyl groups of the phosphines and the isopropyl groups of Trip have been omitted for clarity.

The Mo complex (Fig. 12a) has a very short Mo–Pb bond [2.5495(8) Å], as one would expect for a bond multiplicity of three, and the Mo–Pb–C fragment is practically linear [Mo–Pb–C bond angle is 177.8(2)°] (see Fig. 12b). The two W complexes have very similar structures [32b]. All of these compounds, which are formally analogous to the Fisher-type carbynes (i.e., they are plumbylidynes), are the first derivatives that contain a triply bonded main group element of the sixth row of the periodic table.

As one might expect, the number of complexes containing *three-coordinate lead(II)* is higher than the the number of compounds with the two-coordinate metal. Indeed, Holloway and Melnik [9] cited eight complexes with CN = 3, while our own search of structures incorporated in recent years to the CSD revealed around twenty additional compounds of lead(II) with this coordination number (if the derivatives including metal-metal bonds are also considered). In most of the examples available, the lead coordination sphere is pyramidally configured and displays a stereo-active lone pair.

This low CN is again induced by bulky ligands that, once bound, reduce the coordination volume available around the metal ion and consequently hinder the incorporation of additional donor atoms. The use of the aforementioned bis(trimethylsilyl)amide derivative, $[Pb\{N(SiMe_3)_2\}_2]$ (Fig. 10a), as a stable starting material also opens up synthetic routes for the preparation of compounds with CN = 3.

A representative example is the reaction of this compound with tris(methylsilyl)methanethiol diethyl ether, which affords the dinuclear complex $[Pb(NR_2)(\mu\text{-}SCR_3)]_2$ (R = $SiMe_3$) shown in Fig. 13a [33].

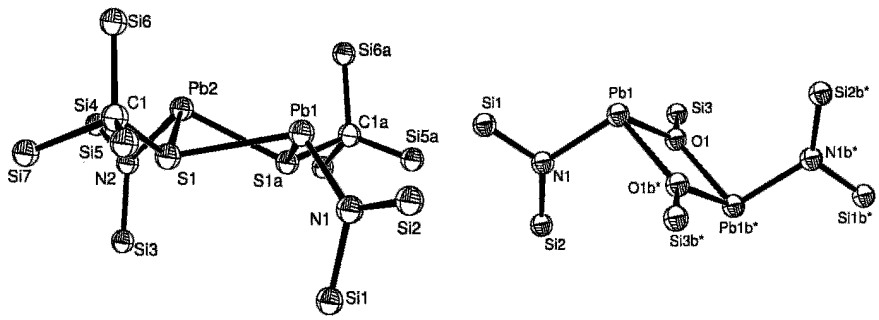
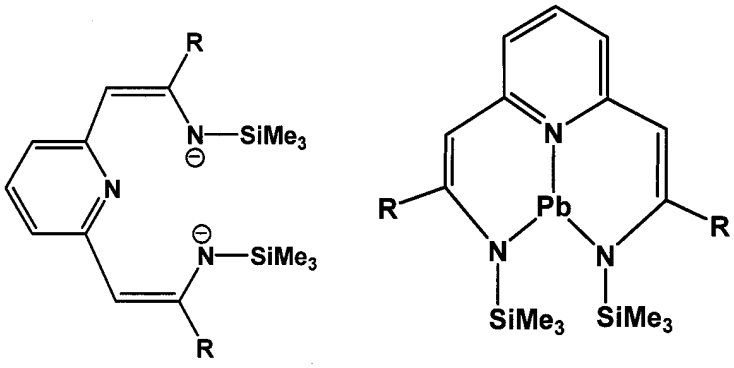


Figure 13. Kernel of (a) $[Pb(NR_2)(\mu\text{-}SCR_3)]_2$ (DEWTII, [33]) and (b) $[Pb(NR_2)(\mu\text{-}OR)]_2$ (VAGKIX, [34]) showing the pyramidal arrangement of the two lead(II) ions (R = $SiMe_3$). The methyl groups have been omitted for clarity.

In addition, the metathesis of $[Pb\{N(SiMe_3)_2\}_2]$ with *tert*-butyl isocyanate produces $[Pb(NR_2)(\mu\text{-}OR)]_2$ [Eq. (3)], which is also a dimeric complex with three-coordinated lead(II) in a trigonal pyramidal environment (Fig. 13b) [34].

$$[Pb\{N(SiMe_3)_2\}_2] + {}^tBuN{=}C{=}O \xrightarrow[\text{pentane, } 25\ ^\circ C]{-Me_3SiN{=}C{=}N^tBu} 1/2\ [Pb\{N(SiMe_3)_2\}\{O(SiMe_3)\}]_2 \qquad \textbf{(3)}$$

Mononuclear complexes can be prepared by reacting the potassium salts of the tridentate bis(1-azaallyl) ligands (Scheme 1, **a**) with $PbCl_2$ in THF.



(a) **(b)**

Scheme 1

Crystallization of this complex from Et_2O afforded crystals of **b** (Scheme 1) with the metal coordinated through the three nitrogen atoms of the ligand. Despite the constraints of the ligand, the geometry at the metal centre is trigonal pyramidal with a SALEP [35].

An exception to this pyramidal stereochemistry can be found in another complex of the ligand (2,6-$Trip_2$-C_6H_3) (see Fig. 9(a)). The complex $[Pb(2,6\text{-}Trip_2\text{-}C_6H_3)Br]_2$ was again the starting material and was reacted with $W(CO)_6$ in THF/hexane solution. The orange solid $[\{W(CO)_4\}_2(\mu\text{-}Br)\{\mu\text{-}Pb(2,6\text{-}Trip_2\text{-}C_6H_3)\}]$ was isolated in low yield (*ca.* 7%) and was studied by X-ray diffraction (Fig. 14) [36].

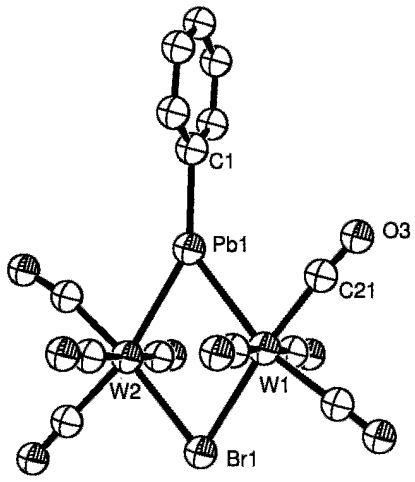


Figure 14. Structure of the complex [{$W(CO)_4$}$_2$(μ-Br){μ-Pb(2,6-$Trip_2$-C_6H_3)}] (YEWGIQ, [36]) showing the trigonal coordination of lead(II). The $Trip_2$ part of the aromatic ligand has been omitted for clarity.

This study showed that the molecule has a plane of symmetry that includes the {PbW_2Br} kernel. The trigonal geometry around the lead ion is very distorted, with two wide W–Pb–C angles (150.04° and 139.78°) and an acute W(1)–Pb(1)–W(2) angle [70.117(8)°].

A final example that displays the more common pyramidal trigonal coordination, but has additional relevance for modelling the Pb(II)-ALAD interaction (see Fig. 8), is the lead(II) complex formed by the tris(2-mercapto-1-phenylimidazolyl)hydroborate ligand {Tm^{Ph}} (see Fig. 15). The {Tm^{Ph}}Li derivative reacts with $Pb(ClO_4)_2{\cdot}xH_2O$ in acetonitrile to give [{Tm^{Ph}}Pb]ClO_4. The Pb(II) in the complex cation is only coordinated to the three sulfur donors of the hydroborate ligand, with the closest interaction being with the perchlorate counterion, which is at a distance of about 3 Å [23]. It is worth noting that not only is the stereochemistry of the metal centre similar to that of Pb-ALAD (Fig. 8), but the average Pb-S bond lengths are also very close to each other (2.7 Å for the {Tm^{Ph}} complex and 2.8 Å for the protein derivative).

However, the preference of {Tm^{Ph}}$^+$ and ALAD to bind lead(II) over zinc(II) (the native metal in the enzyme) is rather different (~500:1 in the first case and ~25:1 in the second), possibly showing the importance of the apoenzyme in this issue.

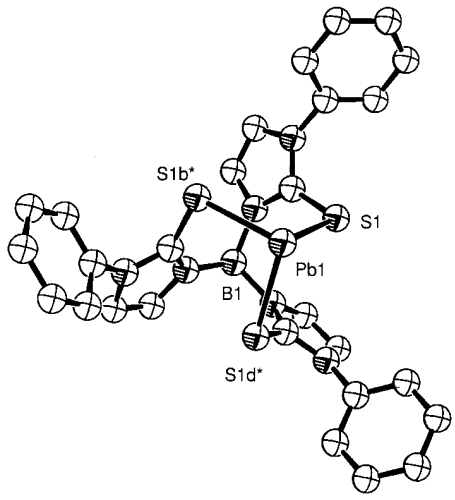


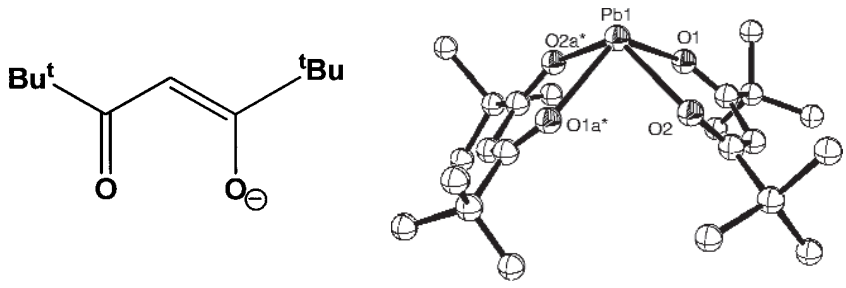
Figure 15. Structure of the $[\{Tm^{Ph}\}Pb]^{+}$ cation (see text) of the $[\{Tm^{Ph}\}Pb]ClO_4$ complex (EBEXEO, [23]) showing the pyramidal trigonal coordination of the metal.

3.2. Coordination number four

Complexes with this coordination number are all hemidirected with two possible ideal stereochemistries: (i) square-pyramidal with the lead ion (or the stereochemically active lone pair) at the apex (Scheme 2, sp) and (ii) pseudotrigonal-bipyramidal with the electron pair hypothetically at one of the equatorial positions (Scheme 2, tp).

sp *tp*

Scheme 2



(a) (b)

Figure 16. (a) Monoanion L^- derived from the deprotonation of 2,2,6,6-tetramethylheptane-3,5-dione. (b) Structure of the complex [PbL_2] (RIMHAW, [37]) showing the square-pyramidal arrangement of the metal and oxygen atoms.

Lead(II) β-diketonates are good representative examples to illustrate PbO_4 kernels. These complexes have recently attracted renewed attention because they can be used as precursors for the development of lead-containing films using metallorganic chemical vapour deposition (MOCVD). Some of these compounds have intermolecular bonding interactions in addition to the four Pb–O bonds formed by the two chelated β-diketonates and therefore have CNs higher than four. However, when the diketonates have bulky substituents, their large volume hinders the formation of such intermolecular contacts and real four-coordinate compounds are formed.

This occurs with the ligand *tert*-butyl β-diketonate (Fig. 16a), which forms a complex with Pb(II) that is described as a square pyramid with the four O atoms at the base and the metal atom (or the SALEP) located at the apex (Fig. 16b) [37]. The Pb–O bond distances (2.30–2.32 Å) are at the lower limit of this parameter in the histogram in Fig. 6.

Lead(II) porphyrinates are good examples of {PbN_4} kernels with a distorted square pyramidal stereochemistry. One interesting example is the complex (5,10,15,20-*tetrakis*-triisopropylsilylethynylporphynato)lead(II), which is prepared by heating a mixture of the porphyrin ligand and $Pb(OAc)_2 \cdot 3H_2O$ under reflux in DMF [38]. The crystal has three independent molecules in the asymmetric unit and these have slightly different structural parameters. As shown in Fig. 17a, in one of the molecules the lead(II) ion and the four nitrogen atoms, which belong to the macrocycle ring, form a distorted square pyramid with the metal atom in an apical position and the N atoms located at the corners of the basal plane. The pyramidal arrangement is more obvious in Fig. 17b, where the peripheral part of the ligand has been omitted for clarity.

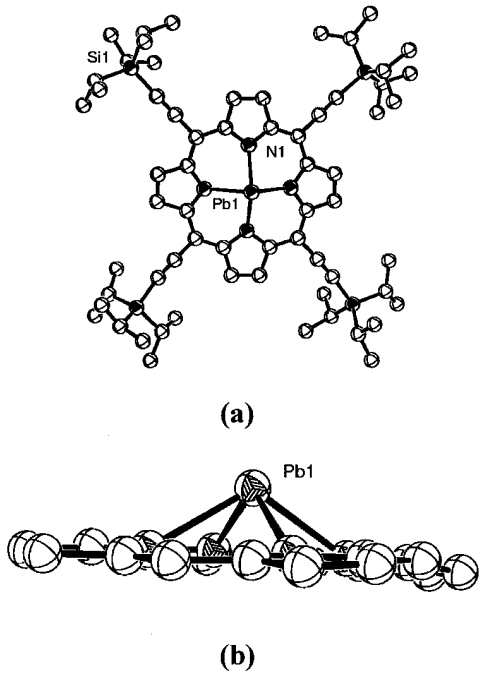


Figure 17. X-ray structure of the (5,10,15,20-*tetrakis*-triisopropylsilylethynylporphinato)lead(II) complex (YIXQEB, [38]) showing (a) one of the three molecules of the asymmetric unit of the crystal and (b) a lateral view of the porphinato-lead(II) kernel.

The Pb–N bond distances vary between 2.388(8) and 2.449(8) Å and are in the range of the shorter distances for this type of bond in coordination compounds (see Fig. 18). The distance between Pb(II) and the least-squares plane through the four basal N atoms is in the range 1.233(4)–1.274(4) Å. The lattice of this solid is built up by the packing of a structural motif consisting of three molecules with a metal-over-metal stack and with the mean planes of the porphyrin rings parallel to one another [38]. The Pb···Pb distance in the same stack is rather long, which rules out metal-metal interaction. The closest Pb···N contacts within a stack (3.15, 3.35 Å) lie in the range of the longest values in the Pb–N bond distance histogram (Fig. 18) but they are shorter than the sum of the van der Waals radii (3.55 Å).

An example of a complex with trigonal-bipyramidal stereochemistry is the phosphorylthiolate complex $[Pb\{2\text{-}(Ph_2P(O)CH_2)C_6H_4S\}_2]$ prepared by reacting $2\text{-}(Ph_2P(O)CH_2)C_6H_4SH$ with lead(II) acetate in methanol [39]. The structure consists of discrete molecules with the two anionic ligands coordinated to the metal through the O and S atoms, each giving a seven-membered chelate ring.

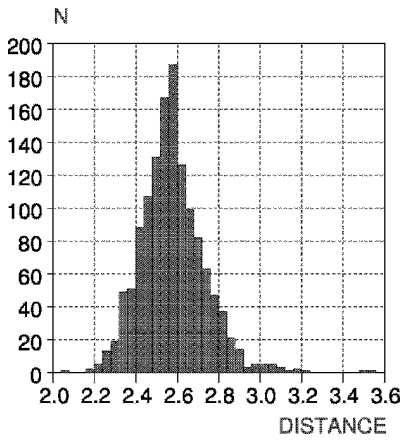


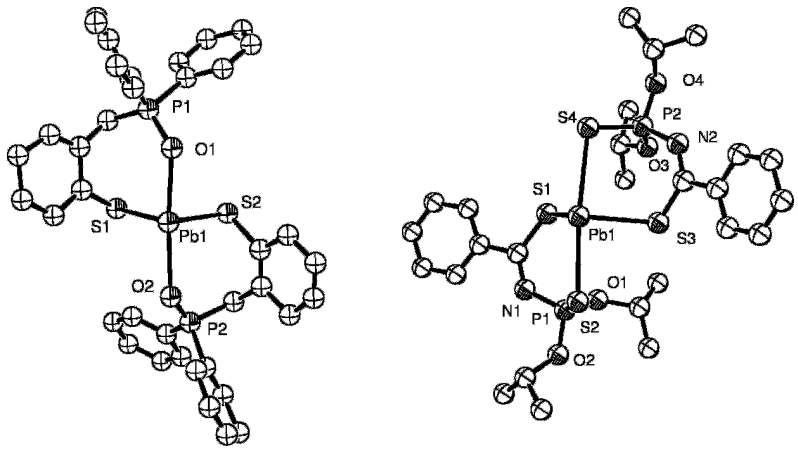
Figure 18. Histogram showing a plot of the number of bonds (N) versus the Pb–N bond distance (DISTANCE) for the complexes included in the CSD [12]. The minimum, maximum and mean values in the X-axis are 2.075, 3.531 and 2.577 Å, respectively.

As shown in Fig. 19a, the two oxygen atoms occupy the axial positions and the two sulphur atoms and presumably a SALEP the equatorial sites. The main distortion of the trigonal geometry occurs at the S–Pb–S angle, which is 94.8° rather than the ideal value of 120°, supporting the presence of the lone pair in the equatorial plane. The VSEPR model indicates that the repulsion between the electron pair follows the sequence:

lone-pair-bonding pair > bonding pair-bonding pair

and so one can expect some narrowing of the S–Pb–S angle to occur under the influence of the SALEP. Additional intra- or intermolecular interactions were not detected.

Recently, particular attention has been paid to sulphur compounds with $\{PbS_n\}$ kernels in the search for single-source precursors for the growth of lead chalcogenide thin films. PbS is an unusual semiconductor and its polycrystalline layers can be used in photoconductive infrared detectors [40]. In some cases, lead(II) dithiocarbamates $\{Pb(dtc)_2\}$ were selected as precursors and this led to an increase in the number of structural studies into these compounds (see ref. 38 and refs. therein). However, $[Pb(dtc)_2]$ are not strictly four-coordinate lead(II) compounds. Although both dtc^- ligands are bidentate (usually anisobidentate) and form four strong Pb–S bonds with a distorted square-pyramidal arrangement, there are also additional weak intermolecular Pb···S interactions that increase the CN up to six.



(a) (b)

Figure 19. Molecular structure of (a) [Pb{2-($Ph_2P(O)CH_2$)C_6H_4S}$_2$] (MAWNIH, [39]) and (b) bis[*N*-(diisopropoxythiophosphoryl)thiobenzamido-*S*,*S*']lead(II) (WITQOF, [41]). The SALEP is presumably above the metal centre pointing toward the observer.

True examples of lead(II) complexes with {PbS_4} kernels are scarce and this peculiarity has been considered a possible reason for the interference of lead in the biological chemistry of zinc (see Section 2 and Ref. 24). Nevertheless, a genuine {PbS_4} kernel occurs in the bis[*N*-(diisopropoxythiophosphoryl)thiobenzamido-*S*,*S*']lead(II) complex shown in Fig. 19b [41].

Once again, the coordination polyhedron of Pb(II) may be defined as a distorted trigonal-bipyramid with the stereochemically active pair occupying one of the equatorial positions. This all-sulphur coordination sphere permits, in this case, an easy comparative analysis of the equatorial and axial Pb–S bond distances. The axial Pb–S bonds [Pb–S(2) and Pb–S(4) in Fig. 19b], with an average distance of 2.92 Å, are longer than the equatorial ones [Pb–S(1) and Pb–S(3), Fig. 19b, average 2.70 Å] although both are well within the range for this type of bond in lead(II)-sulphur complexes (see Fig. 20). This axial elongation is common in trigonal-bipyramidal complexes of non-transition elements [41].

In the preceding examples the corresponding geometries are easily defined, but very often the deformation of the coordination sphere makes the stereochemistry of this coordination number hard to describe briefly and precisely. Expressions such as "strongly distorted" trigonal-bipyramid, square-pyramid or tetrahedral are used in these cases, although these terms are not always correctly applied. Structures in which the widest X–Pb–Y bond angle is

less than 140° are probably best described as square-pyramidal with a certain degree of distortion.

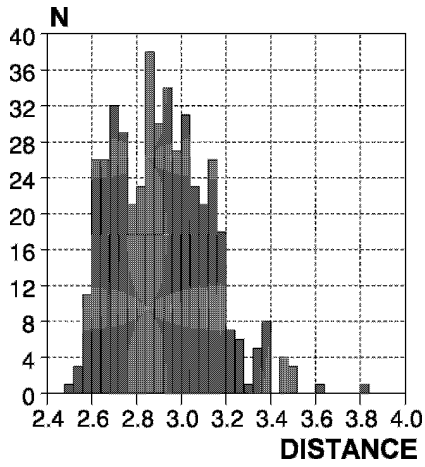
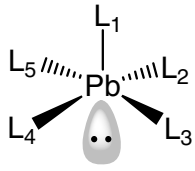


Figure 20. Histogram showing a plot of the number of bonds (N) versus the Pb–S bond distance (DISTANCE) for the complexes included in the CSD [12]. The minimum, maximum and mean values in the X-axis are 2.511, 3.805 and 2.916 Å, respectively.

3.3. Coordination number five

There are not many complexes with this coordination number and all the known examples have hemidirected coordination geometries. In cases where there are six electron pairs around an atom (one lone pair and five bonding), the VSEPR model predicts an octahedral distribution that produces an apparent square-based pyramidal shape (Scheme 3).



Scheme 3

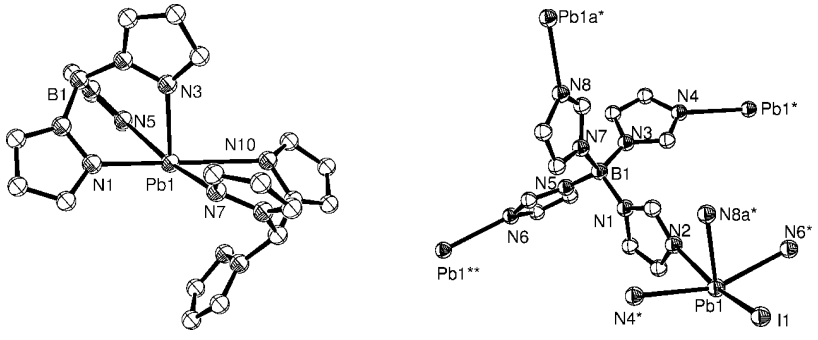
The SALEP occupies a greater volume than the bonding pairs and this means that the L_1–Pb–L_n (n = 2, 3, 4 and 5) angles are usually narrower than the ideal 90° angle and the pyramid always shows some distortion.

Some examples of mononuclear complexes with this type of coordination have been prepared and their structures fully identified. For example, Reger et al. worked on a series of lead(II) derivatives of tris(pyrazolyl)methane {HC(3,5-

$Me_2pz)_3\}$ and tris(pyrazolyl)borate $\{HB(3,5\text{-}Me_2pz)_3\}^-$ (pz = pyrazolyl ring) ligands and prepared the complex $[Pb\{HB(3,5\text{-}Me_2pz)_3\}\{HC(3,5\text{-}Me_2pz)_3\}](BF_4)$ [42]. The structure of the $[Pb\{HB(3,5\text{-}Me_2pz)_3\}\{HC(3,5\text{-}Me_2pz)_3\}]^+$ monocation (see Fig. 21a) shows that the lead(II) ion is five-coordinate with a tridentate $\{HB(3,5\text{-}Me_2pz)_3\}^-$ anion and a bidentate $\{HC(3,5\text{-}Me_2pz)_3\}$ neutral ligand.

The distorted square-based pyramid has two nitrogen atoms from each ligand [N(1), N(5) from the $HB(3,5\text{-}Me_2pz)_3$ ligand and N(7), N(10) from the $HC(3,5\text{-}Me_2pz)_3$ ligand] occupying the basal positions and the N(3) atom of the tris(pyrazolyl)borate ligand at the apex [42]. The remaining nitrogen donor atom of the tris(pyrazolyl)methane ligand is 3.19 Å away from the lead(II) centre, suggesting either a very weak interaction with the metal (see Fig. 18) or simply demonstrating the structural rigidity of the $HC(3,5\text{-}Me_2pz)_3$ ligand. As one would expect in the presence of a SALEP, the length of the bond *trans* to this pair [Pb(1)–N(3), 2.375(7) Å] is shorter than those of the bonds located closer to the void where the pair hypothetically occurs [Pb(1)–N(X), X = 1, 5, 7, 10, d_{Pb-N} = 2.430(7)–2.827(7) Å].

The $[Pb\{B(Im)_4\}]NO_3(xH_2O)$ complex (see Section 2 and Fig. 2), when suspended in an aqueous solution of NaI, exchanges nitrate for iodide and affords $[Pb\{B(Im)_4\}I]$ crystals. Unlike the nitrate complex, iodide coordinates to the metal to give a Pb–I bond. This bond increases the coordination number from four (Fig. 2) to five (Fig. 21b) even though the stereochemistry of the iodide complex remains hemidirected.



(a) **(b)**

Figure 21. Structure of the (a) $[Pb\{HB(3,5\text{-}Me_2pz)_3\}\{HC(3,5\text{-}Me_2pz)_3\}]^+$ cation (RIBSUQ, [42]) and (b) $[Pb\{B(im)_4\}I]$ (im = imidazolyl ring) complex (IQOBIZ, [14]) showing the five-coordinate lead(II) ion with a distorted square-pyramidal coordination sphere. In (a) the methyl groups of the ligand have been omitted for clarity.

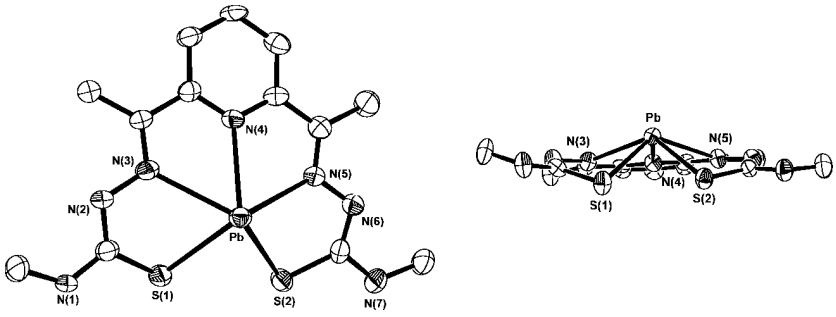


Figure 22. The complex of Pb(II) with 2,6-diacetylpyridine bis(4-*N*-methylthiosemicarbazone) showing (a) the N_3S_2-coordination of the ligand and (b) the "umbrella-like" distorted pentagonal coordination geometry. Hydrogen atoms have been omitted for clarity [43b].

Two complexes with an alternative stereochemistry have recently been isolated and structurally characterized [43]. Both are compounds of the 2,6-diacetylpyridine bis(thiosemicarbazone) ligand or its 4-*N*-methyl derivative.

The crystal structure of the latter complex consists of discrete molecules (Fig. 22a). The complex has a distorted "umbrella-like" pentagonal pyramidal structure with the N_3S_2-donor set of the ligand at the base and the metal (or the SALEP) at the apex. The lead(II) ion is located 1.10 Å above the least-squares plane through the five basal atoms (Fig. 22b). Curiously, the diorganolead(IV) complexes of 2,6-diacetylpyridine bis(thiosemicarbazone) have the metal practically inside the void of the ligand (only 0.0027 Å above the least-squares plane through the N_3S_2 atoms) [43a]. It remains to be seen whether this change is due to the presence of a SALEP in the lead(II) complex or to the smaller size of the metal centre in the lead(IV) derivative.

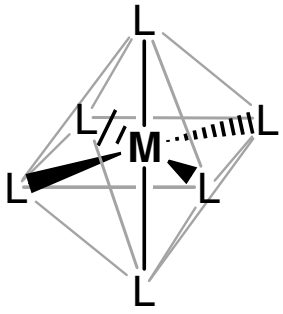
3.4. Coordination number six

In all the preceding CNs the complexes found in the literature all proved to have hemidirected geometries, which is consistent with the previous findings of Glusker et al. [10]. As indicated before, CN 6 (like CN 7 and 8) allows both holodirected and hemidirected structures. Nevertheless, a search of the CSD shows that the number of complexes with a holodirected arrangement of ligands is far greater than the hemidirected examples (69% and 31%, respectively, for lead(II) complexes with this CN [10]).

Only fifteen mononuclear non-polymeric complexes of Pb(II) with CN 6 were included in the review by Holloway and Melnik [9]. The literature that has

appeared since 1996 (the last year that was partially included in the aforementioned review) has contained more than thirty further compounds of this type and these mainly include macrocyclic or bulky ligands. The halide and carboxylate derivatives with this CN very often adopt polymeric arrangements.

It is well known that complexes with this coordination number and six bonding pairs usually adopt a more or less distorted octahedral geometry (Scheme 4).

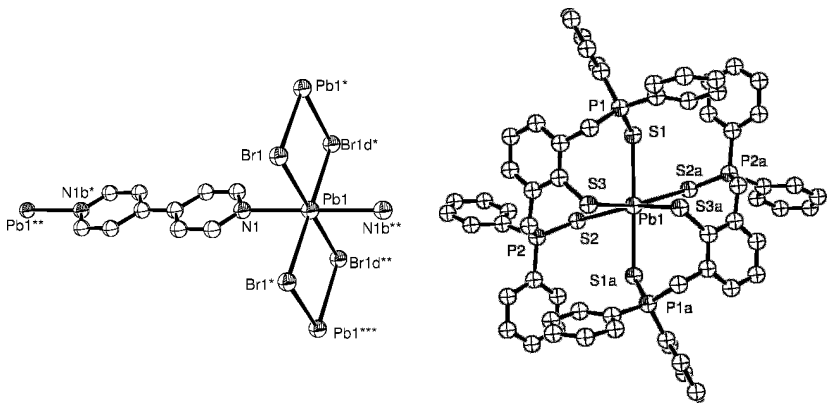


Scheme 4

The structures in which the ligands lie at the vertices of a trigonal prism are much less common. In fact, examples of holodirected lead(II) complexes with the latter geometry were not found in the literature.

There are, however, numerous holodirected compounds with an octahedral geometry, with the most regular structures probably being those exhibited by the isolated hexahaloplumbate(II) anions $[PbX_6]^{4-}$. For example, the $[PbCl_6]^{4-}$ ion in $Cs_4[PbCl_6]$ is an almost regular octahedron, with Pb–Cl distances ranging from 2.882(3) to 2.889(3) Å and bond angles differing from the ideal value (90°) by less than 0.42° [9]. In the alkylammonium salt $(ClCH_2CH_2NH_3)_4[PbCl_6]\cdot 2(ClCH_2CH_2NH_2\cdot HCl)$, the discrete centrosymmetric $[PbCl_6]^{4-}$ anion is octahedral with a small distortion due to the presence of hydrogen bonds involving the Cl ligands and the ammonium cation. These interactions cause a slight modification of the Pb–Cl bond lengths and the Cl–Pb–Cl bond angles [44]. It is worth noting that that these holodirected arrangements are consistent with rule *(ii)* discussed above (see Section 2).

Octahedral holodirected haloplumbate(II) arrangements are also present in extended chains with one, two or three bridging halides, or in two- or three-dimensional networks, which are found in certain mixed organic-inorganic perovskites that have interesting optical properties. For example, the 3D perovskite organic-inorganic hybrids, $APbX_3$, consist of an extended three-dimensional arrangement of the all-corner-sharing PbX_6 octahedra with the cation A^+ (a monoammonium organic cation) filling the holes between the octahedra [45].



(a) **(b)**

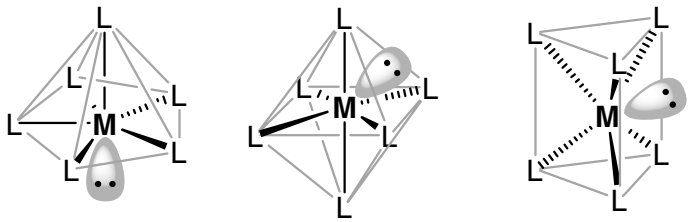
Figure 23. Two holodirected octahedral complexes: (a) $[PbBr_2(bipy)]_n$ (XEGRAC, [46]) and (b) $[Pb\{2,6\text{-}(Ph_2P(S)CH_2)_2C_6H_3S\}_2]$ (MAWPAB01, [39, 47]).

Regular holodirected octahedral polymeric structures also occur in some adducts of lead(II) halides with organic bases. One example is the $[PbBr_2(bipy)]$ complex formed by the digestion of $Pb(OOCCH_3)_2$, NaBr and 4,4'-bipyridine (bipy) in water at 393 K in a digestion bomb. According to an X-ray study [46], this compound has an octahedrally coordinated metal centre, with four bridging Br ligands and two bridging bipy molecules (see Fig. 23a), giving a two-dimensional layer in the *bc* plane. The octahedral coordination is basically distorted by the difference between the Pb–Br bond distance [2.9992(18) Å] and that of the *trans* Pb–N bonds [2.659(14) Å].

Holodirected six-coordination can also be achieved when lead(II) is coordinated to polydentate ligands but in these cases the complexes are usually far from octahedral symmetry because of the constraints imposed by the ligand geometry. The molecular structure of the complex $[Pb\{2,6\text{-}(Ph_2P(S)CH_2)_2C_6H_3S\}_2]$ is shown in Fig. 23b and it can be seen that each monodeprotonated tridentate ligand coordinates to the metal through the thiolate sulphur and the two P=S groups [39]. If both strong and weak bonding interactions are taken into account, this compound can be considered an example of severely distorted holodirected octahedral stereochemistry. There are three types of Pb–S bonds: (i) the shortest ones (2.675 Å) comprise the S atoms of the deprotonated thiolate groups [S(3) and S(3a), Fig. 23b]; (ii) the medium ones (2.987 Å) involve the S atoms of two of the P=S groups [P=S(1) and P=S(1a)]; and (iii) the longest ones (3.378 Å) correspond to the secondary interactions

between the metal centre and the remaining two P=S groups [P=S(2) and P=S(2a)]. The latter distance is rather long but is significantly shorter than the sum of the van der Waals radii (3.80 Å). The structural distortion also affects the bond angles, which range from approximately 72 to 98° [47] and are significantly different from the ideal value (90°). It is worth noting that, as in the preceding cases, the donor atoms are also soft and so rule (*ii*) (see Section **2**) applies (holodirected stereochemistry). However, in this case the connectivity between the donor atoms (tridentate ligand) should favour a hemidirected arrangement [rule (*i*), Section **2**].

If the 6s pair is stereochemically active, then the six-coordinate complexes have seven electron pairs – six of which are bonding pairs – and so three stereochemical arrangements can be expected with similar stabilities: a pentagonal bipyramid (pb), a capped octahedron (co) and a capped trigonal prism (ctp) (see Scheme 5). There are some clear examples of hemidirected six-coordinate lead(II) complexes that adopt the first two stereochemistries but many compounds with a CN of 6 also exist with a highly irregular distribution of ligands and these are not easily identifiable with any usual stereochemistry.



pb *co* *ctp*

Scheme 5

An example of a lead(II) centre with the *pb* coordination and a SALEP has been discussed above in relation to the structure of the compound $Pb_5(SiO_4)(VO_4)_2$ (Fig. 3).

A derivative of 2,6-diacetylpyridine bis(semicarbazone) (DAPSC), namely $[PbCl(DAPSC)]NO_3$, is another complex with a pb structure that was identified early on. An X-ray diffraction study on this complex showed that the DAPSC molecule is pentadentate and its set of $\{N_3O_2\}$ donor atoms occupy the equatorial plane of a hypothetical pentagonal bipyramidal coordination sphere with the chloro ligand and the metal pair at the axial positions (Fig. 24) [48]. The Pb–Cl bond distance [2.707(4) Å] is significantly shorter than in $Cs_4[PbCl_6]$ (*ca.* 2.9 Å, *vide supra*), as one would expect if this bond in the present complex is *trans* to the stereochemically active lone pair.

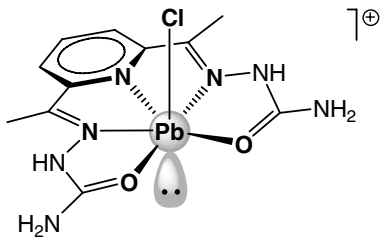


Figure 24. Structure of the $[PbCl(DAPSC)]^+$ cation of the complex $[PbCl(DAPSC)]NO_3$ [48].

It is worth noting that DAPSC is closely related to 2,6-diacetylpyridine bis(4-*N*-methylthiosemicarbazone) (see Fig. 22) although, in the present complex, there is one additional chloro ligand. It is also noteworthy that, even though one ligand is soft (Cl^-), because DAPSC is a multidentate coordination entity with five hard donor atoms, the final stereochemistry of the complex is hemidirected.

It is also interesting to observe that the distances from the lead(II) ion to the closest O atoms of the nitrate counterion (2.973 and 3.095 Å) are very long but are less than the sum of the van der Waals radii (3.50 Å). Taking these weak interactions into account, the coordination number of Pb(II) in the complex increases to eight, with the weaker bonds, as usual, close to the void in the coordination sphere, where the SALEP is supposed to occur.

In the search for systems that mimic the bimetallic cores of phosphatase enzymes, some bimetallic complexes containing Zn(II) and Pb(II) ions have been prepared [49]. In order to achieve the best mimetic behaviour, a compartmental ligand L^{2-} with two non-equivalent metal-binding sites was selected. This ligand (Fig. 25a) has two phenol groups linking one "salen"-like fragment (left side of the L^{2-} formula in Fig. 25a) and one "saldien"-like fragment (right side of the formula in the figure) [salen = *N*,*N*'-ethylenedisalicylideneaminate and saldien = *N*,*N*'-1,5-(3-azapentylene)disalicylideneaminate].

One of the synthesized complexes, $[ZnPbL(OH)]ClO_4$, has a dimeric structure formed by two dinuclear $\{ZnPbL(OH)\}^+$ units, which are bridged by the hydroxo oxygens O(3) and O(3)* (Fig. 25b; note that, in order to give a clearer understanding of the dimeric arrangement, the unit $\{ZnPbL(OH)\}^+$ is shown along with one μ-OH group and the two metal centres belonging to the other unit). In each $\{ZnPbL(OH)\}^+$ unit the Zn(II) and Pb(II) ions are bridged by the phenolic oxygens and the first metal resides in the "salen" site, while the second one occupies the "saldien" fragment. As a result, Pb(II) is coordinated to the $\{N_3O_2\}$ set of donor atoms from this fragment and to the O atom of the bridging hydroxide. It can be seen from Fig. 25b that the geometry around the Pb(II) centre is best described as a pentagonal pyramid with the O of the μ-OH

group axial and the L donor atoms basal. The bond distance of the Pb–O(3) bond [2.276(5) Å] is significantly shorter than the Pb–L bonds [2.551(8)–2.636(6) Å], as one would expect from the position of these bonds in relation to the SALEP; Pb–O(3) is *trans* to this pair and the Pb–L bonds are closer to the void. Both the hard character of the donor atoms and the multidentate behaviour of L^{2-} favour the adopted hemidirected stereochemistry.

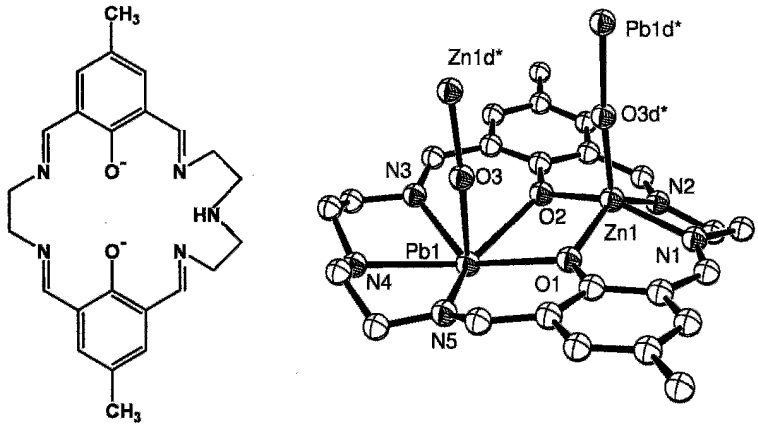


Figure 25. (a) Formula of the macrocyclic ligand L^{2-} and (b) partial representation of the $[ZnPbL(OH)]_2^{2+}$ cation of the $[ZnPbL(OH)]ClO_4$ complex (JUJQIO, [49]).

The capacity of this type of compound to hydrolyze tris(*p*-nitrophenyl) diphosphate into bis(*p*-nitrophenyl) phosphate in dimethyl sulfoxide was explored using ^{31}P NMR and visible spectroscopy. It was concluded that the {Zn(OH)Pb} core is essential for efficient hydrolysis [49].

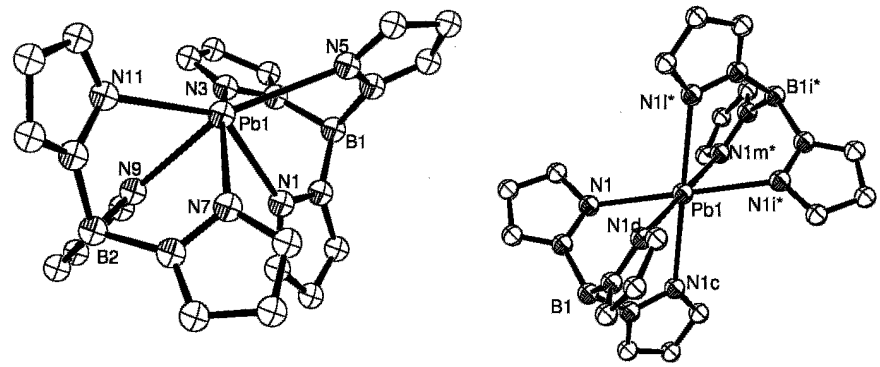
Other characteristics of the ligand, in addition to the nature of the donor atoms, can also influence the choice of a holo- or hemidirected stereochemistry. This situation is well illustrated by the complexes $[Pb\{HB(pz)_3\}_2]$ and $[Pb\{HB(3,5\text{-}Me_2pz)_3\}_2]$, where $HB(pz_3)^-$ is the anion tris(pyrazolyl)borate and $HB(3,5\text{-}Me_2pz)_3^-$ is its 3,5-dimethylpyrazolyl analogue [50a] (see also **3.3.**). In the former complex, and according to the rules discussed previously, the presence of connected N donor atoms should lead to complexes with a SALEP. In fact, this is the situation in $[Pb\{HB(pz)_3\}_2]$, in which the coordination geometry around the metal centre can be described as a *capped octahedron*, with the active electron pair located over the capped triangular face, which is delimited by the atoms N(3), N(5) and N(11) (see Fig. 26a). The Pb–N bond

distances in this face (average 2.726 Å) are, as expected, longer than the other three (average 2.487 Å) due to the proximity of the former to the pair position.

The replacement of this ligand by a bulkier one with two methyl groups at the 3,5-positions of each pyrazolyl ring, gives the complex [Pb{HB(3,5-$Me_2pz)_3\}_2$], which also has a six-coordinate lead(II) ion. However, in this case the complex has an octahedral trigonally distorted geometry (Fig. 26b). The metal sits on a centre of inversion and the lone pair is stereochemically inactive even though the set of donor atoms is very similar to that in [Pb{$HB(pz)_3\}_2$]. This structural difference has been attributed to steric effects introduced by the methyl substituents present in the $HB(3,5\text{-}Me_2pz)_3$ ligand. Similar results were obtained on using tris(pyrazolyl)methane molecules as ligands [50b].

It is clear that the application of aprioristic arguments, such as those derived from the aforementioned rules, should be used carefully because several influential factors can be working simultaneously, easily leading to incorrect conclusions. These difficulties are clearly greater when the ligand has both soft and hard donor atoms, and it is precisely this type of ligand that is relevant because lead(II) is a borderline acid according to the HSAB principle.

Although the hemidirected structures mentioned above can be structurally identified with one of the archetypical models, bp or co (see above), this is not always the case. As pointed out before, many structures with CN 6 and a SALEP have a rather irregular arrangement of the atoms in the coordination sphere, making it difficult to describe them as anything but "irregular".



(a) **(b)**

Figure 26. X-ray structure of the tris(pyrazolylborate) complexes (a) [Pb{$HB(pz)_3\}_2$] (VOTVUV) and (b) [Pb{$HB(3,5\text{-}Me_2pz)_3\}_2$] (VOTWAC) showing the hemi- and holodirected arrangement of the ligands, respectively [50a].

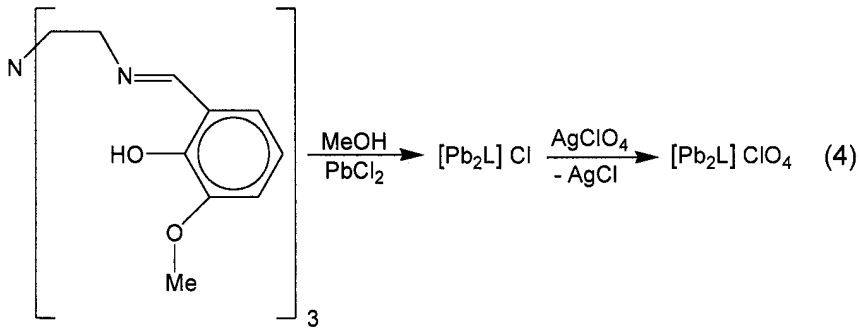
3.5. Coordination numbers higher than six

In lead(II) coordination chemistry there are many examples of complexes with coordination numbers from seven up to twelve. In this section only a few representative complexes have been selected for discussion. Interested readers who require a more detailed treatment of this topic are referred to more comprehensive literature [5-11].

As already mentioned, *CN seven* includes complexes with holo- and hemidirected structures and these mainly contain O and N donor atoms in the coordination sphere – although in some cases they also contain halide anions. Surprisingly, the proportion of hemidirected versus holodirected arrangements of ligands increases for this CN with respect to CN 6. More specifically, three out of four lead(II) seven-coordinate complexes adopt the hemidirected arrangement [10], although one would expect the opposite to happen; [note that higher CNs should lead to higher inter-ligand repulsion, making the stereochemical activity of the 6s pair more difficult according to rule *(ii)*].

In theory, holodirected coordination complexes with CN 7 would, in the absence of steric restrictions, probably adopt a more or less distorted pentagonal bipyramidal or monocapped octahedral arrangement of ligands around the lead(II) centre. For hemidirected derivatives, the hexagonal pyramidal distribution should be compatible with the presence of a SALEP opposite the apical position of the pyramid. In fact, Holloway and Melnik mainly found these arrangements in their review of lead(II) seven-coordinate complexes [9]. Several variations on these geometries are also possible and these are illustrated by the examples described below.

The reaction of the tripodal Schiff base ligand tris[2-(3-methoxysalicylideneamino)ethyl]amine (H_3L) with lead(II) chloride and triethylamine in refluxing methanol afforded $[Pb_2L]Cl$ in good yield [see Eq. (4)]. This complex was transformed by anion metathesis into $[Pb_2L]ClO_4$, which was studied by X-ray diffraction [51].



(4)

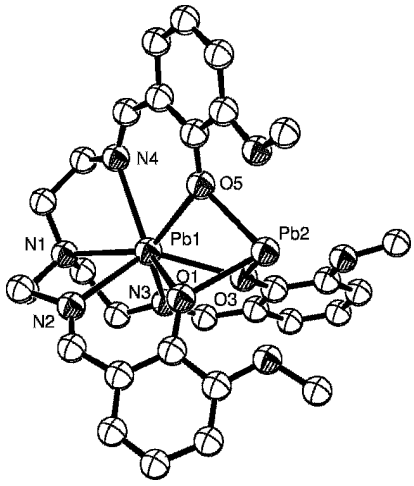
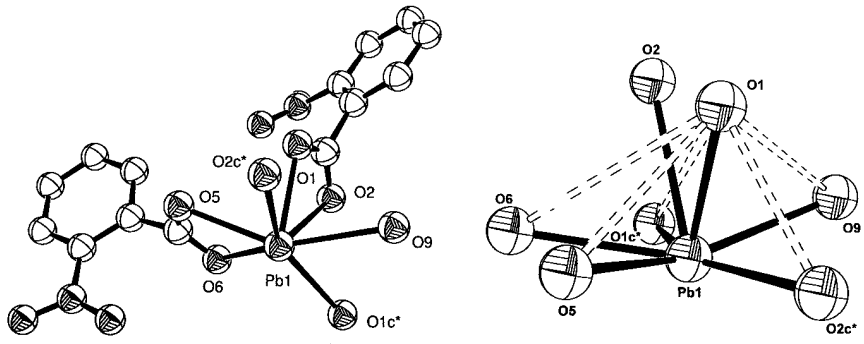


Figure 27. Structure of the $[Pb_2L]^+$ cation of the $[Pb_2L]ClO_4$ complex (JIQLUQ, [51]) (H_3L = tris[2-(3-methoxysalicylideneamino)ethyl]amine).

The $[Pb_2L]^+$ cation contains two lead(II) centres {Pb(1) and Pb(2)} with dissimilar coordination arrangements. Pb(2) is bound to the L^{3-} ligand through the three phenolate oxygen atoms [O(1), O(3) and O(5)] and these form a trigonal pyramidal coordination sphere, possibly with a SALEP (Fig. 27). The donor atom arrangement around the seven-coordinate Pb(1) is best described as a distorted monocapped octahedron in which the presence of the lone pair is not evident.

Another seven-coordinate complex, $[Pb(C_8H_4NO_4)_2(H_2O)]$ ($C_8H_4NO_4$ = *o*-nitrobenzoate), was recently prepared by heating lead acetate, *o*-nitrobenzoic acid and water under hydrothermal conditions [52]. The two carboxylate anions chelate to the lead(II) ion and one of them also bridges between the metal centres to form 1-D coordination polymers. The water molecule [O(9)] is also coordinated and is 2.676 Å from the lead ion. If one considers all of the Pb–O bonds with a bond distance below 2.8 Å, the complex has CN 7 (see Fig. 28a) and a coordination sphere with an evident void and a grossly distorted edge-capped pentagonal pyramidal geometry. This geometry is shown in Fig. 28b, with the apex selected on the assumption that the stereochemically active lone pair is located below the base defined by O(1c*), O(2c*), O(5), O(6) and O(9). If this is the case, then the Pb–O bond distance opposite to it, i.e., [Pb–O(1)], should be the shortest bond distance, which was found to be the case [2.421(4) Å].

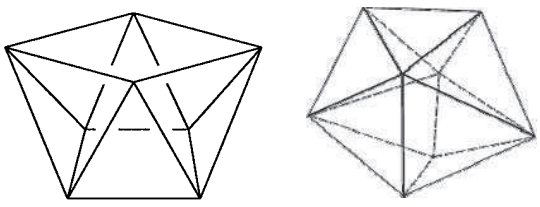


(a) **(b)**

Figure 28. (a) Pb(II) coordination environment in the complex $[Pb(C_8H_4NO_4)_2(H_2O)]$ ($C_8H_4NO_4$ = *o*-nitrobenzoate) (QOZBOW, [52]); (b) detail of the coordination sphere showing the distorted edge-capped pentagonal pyramidal geometry.

Lead(II) complexes with *coordination number 8* also exhibit holo- and hemidirected geometries. Although an earlier review of the CSD [10] indicated a predominance of the former system (*ca.* 88% of the eight-coordinate lead(II) compounds included in the database at that time), the recent literature contains a significant number of complexes with a hemidirected stereochemistry.

The two most important eight-coordinate geometries in metal complexes are square antiprismatic (Scheme 6, sa) and trigonal dodecahedral (Scheme 6, td), probably because these two arrangements alleviate the repulsion between the donor atoms. Nevertheless, other geometries, such as cubic, bicapped trigonal prismatic or hexagonal pyramidal, are also possible, although they are less frequent.



sa ***td***

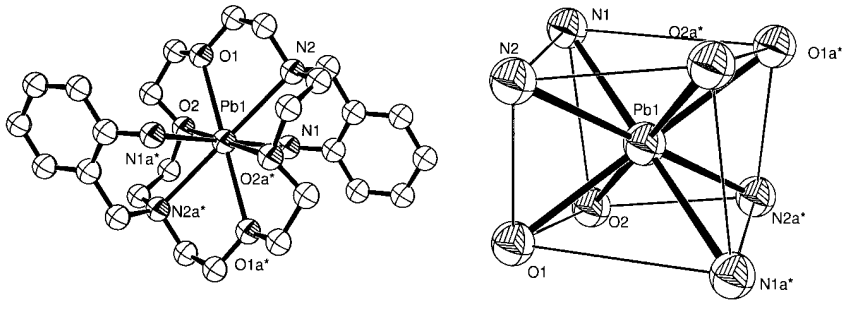
Scheme 6

All of these geometrical arrangements of ligands have been found in lead(II) complexes and other arrangements have also been observed, such as a capped pentagonal pyramid [53] or a tricapped square pyramid [54].

A few examples of close-to-cubic holodirected stereochemistries have been observed [55]. One of these examples [55a] is the lead(II) complex $[PbL](SCN)_2 \cdot H_2O \cdot EtOH$, where L is a macrocycle derived from 4,13-diaza-18-crown-6. The structure shown in Fig. 29a is the $[PbL]^{2+}$ cation containing the lead(II) centre coordinated to the $[O_4N_4]$ donor set of the lariat ether (a crown ether macrocycle with side-arms appended to the macro-ring, which in this case are two 2-aminobenzyl groups that also coordinate to the metal).

The coordination polyhedron (Fig. 29b) can indeed be described as a distorted cube, with significant differences in the lengths of the edges and with the mean deviation from planarity of the six faces in the range 0.1061–0.1849 Å [55a].

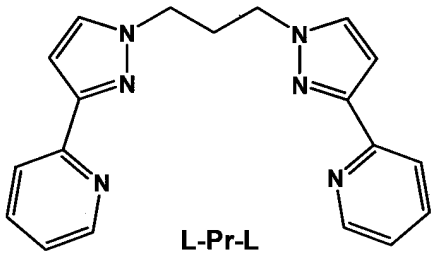
Other examples of this highly regular eight-coordination are the two cationic complexes that contain a derivative of *p-tert*-butyl calix[4]arene as a ligand. In these cases, the $[O_8]$-coordination sphere forms the corners of a distorted cube that is tetragonally compressed along the calixarene axis [55b]. The chelating ligand 3-(2-pyridyl)pyrazole (L) also forms two eight-coordinated complexes with lead(II), $[PbL_4]\{(MeO)_2PO_2\} \cdot H_2O$ and $[PbL_4](PF_6)_2$. The former has a square prismatic geometry while the latter has a similar structure but is distorted towards a square antiprismatic arrangement. The difference appears to be associated with the stabilization of the former complex through hydrogen bonding between L and the dimethylphosphate anion [55c].



(a) **(b)**

Figure 29. (a) Structure of the $[PbL]^{2+}$ cation of the $[PbL](SCN)_2 \cdot H_2O \cdot EtOH$ complex (XOTFER, [55a]); (b) detail of the lead(II) coordination sphere.

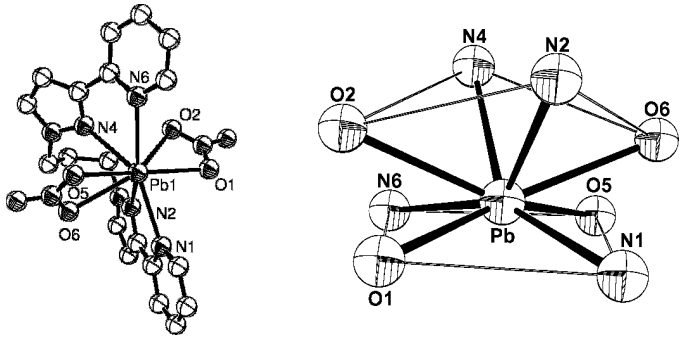
It is worth noting that the tetradentate chelating ligand 1,3-bis[3-(2-pyridyl)pyrazol-1-yl]propane (Scheme 7, L–Pr–L), formed by linking two 3-(2-pyridyl)pyrazole ligands (L in the preceding example) with a flexible propyl chain, forms the complex $[Pb(L–Pr–L)(NO_3)_2]$ that also contains eight-coordinated lead(II) but shows significant stereochemical differences in comparison to $[PbL_4]\{(MeO)_2PO_2\}\cdot H_2O$ or $[PbL_4](PF_6)_2$ [56].



Scheme 7

Besides the four Pb–N bonds formed by the (L–Pr–L) ligand, the CN 8 is completed by the four Pb–O bonds formed by the two chelated nitrate ligands (Fig. 30a).

The coordination geometry of this complex (Fig. 30b) is rather irregular but can be described as a distorted square antiprism, although one of the prism planes [the "plane" in Fig. 30b formed by O(2), N(2), N(4) and O(6)] deviates significantly from a planar arrangement. On the other hand, the more regular plane [O(1), N(1), O(5), N(6)], which is closer to the metal centre, has a more "open" atomic arrangement (the average interatomic separation between atoms along the four edges of the plane is 3.77 Å whereas it is only 3.24 Å in the upper "plane"). This situation leads to a gap in the coordination sphere that can be attributed to a SALEP through the lower plane [56].

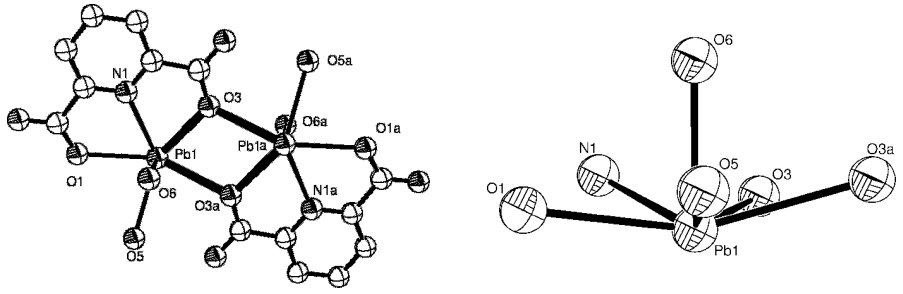


(a) **(b)**

Figure 30. (a) Structure of $[Pb(L–Pr–L)(NO_3)_2]$ (GIMPUN, [56]); (b) detail of the coordination geometry of the metal centre.

An interesting additional example is provided by the complex $Pb_2(dipic)_2(H_2dipic)_2(H_2O)_6$ (H_2dipic = dipicolinic acid or pyridine-2,6-dicarboxylic acid), which was obtained by the reaction under slow diffusion conditions of a solution of lead(II) nitrate or acetate with the corresponding acid. The structure of this complex was studied by X-ray diffraction for the first time in 1979 [57a]. It was belive at the time that the representative formula for the compound was $[Pb_2(dipic)_2(H_2O)_4]\cdot 2H_2dipic\cdot 2H_2O$, with the lattice formed by molecules of the dimeric complex $[Pb_2(dipic)_2(H_2O)_4]$ (Fig. 31Aa) as well as crystallization molecules of H_2dipic and water (not included in the figure). The two Pb(II) centres in the dimer are bridged by carboxylate oxygen atoms to give rhombohedral Pb_2O_2 arrangements (see the four bolder lines in Fig. 31Aa). The $dipic^{2-}$ ligands are tridentate and the coordination sphere around each metal centre is completed to six by the bridging Pb–O bond and the oxygen atoms [O(5) and O(6)] from two coordinated water molecules. The distances between each lead(II) ion and the possible donor atoms of the crystallization molecules were considered too long to bring about any significant bonding interaction.

On the basis of this interpretation, each lead(II) ion is six-coordinate with the donor atoms (pentagonal pyramidal geometry) hemidirected and the SALEP occupying the position below the base of the pyramid, opposite to O(6) (see Fig. 31Ab).



(a) **(b)**

Figure 31A. (a) Crystal structure of the complex $Pb_2(dipic)_2(H_2dipic)_2(H_2O)_6$ (PYCXPB) according to [57a] showing the arrangement of the dimer $[Pb_2(dipic)_2(H_2O)_4]$ and the Pb_2O_2 fragment in bolder lines (the two H_2dipic and two H_2O crystallization molecules have been omitted for clarity); (b) detail of the coordination geometry around the metal centre.

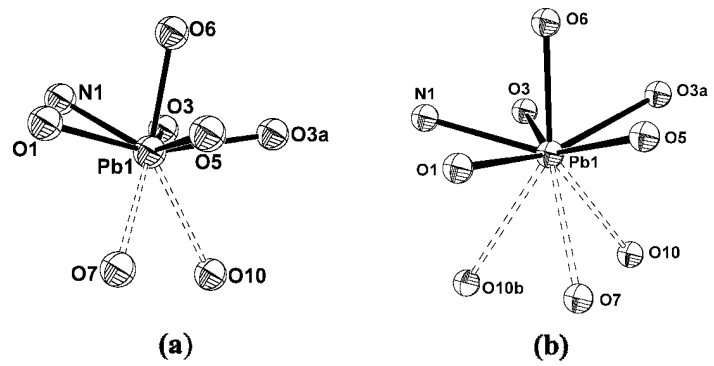


Figure 31B. Detail of the coordination sphere of the metal centre in the complex $Pb_2(dipic)_2(H_2dipic)_2(H_2O)_6$ (PYCXPB01) according to [57b], showing the donor atoms at a distance of less than (a) 2.8 Å and (b) 3.1 Å from Pb(1). The weaker bonding interactions are represented as dashed lines.

When the structure of this complex was studied again by X-ray diffraction in 2003 [57b] the interpretation was different. This illustrates the difficulties in establishing the limit of the coordination sphere in some lead(II) complexes (see Introduction). In this recent study, it was found that the oxygen atom O(7) from one of the water molecules and the oxygen atom [O(10)] from one of the H_2dipic molecules, both considered crystallization molecules in the former study [57a], are in fact located near to the metal centre [3.031(5) and 3.073(5) Å, respectively]. If these distances, which are clearly shorter than the sum of the van der Waals radii (3.50 Å), are considered indicative of significant bonding interactions, then the coordination number increases to eight (see Fig. 31Ba, bonds in dashed lines).

It must be noted that these two new bonds do not rule out the presence of a SALEP, because the long bond distances associated with the Pb(1)–O(7) and Pb(1)–O(10) bonds may be due to the presence of such a pair, which would prevent a closer interatomic approach. Even when the "inter-dimer" stacking is considered and a new Pb(1)–O(10b) contact (3.277 Å) is taken into account, thus increasing the CN up to nine (Fig. 31Bb), this new interaction is once again located in the void beneath the base of the pentagonal pyramid.

Holloway and Melnik [9] found five *nine-coordinate* complexes in their review of the CSD up to the end of 1995 and the major journals up to the middle of 1996. Since then, no less than twenty five additional examples have been added to the database. In general, the complexes with this high CN are formed by macrocyclic (or polydentate) ligands with N or O donor atoms, although some rare exceptions also incorporate S donor atoms. The metal-ligand bond distances usually cover a broad range of values, the longest of which are clearly

only indicative of weak bonding interactions. As pointed out before (see Section 2), although Glusker et al. [10] only found examples of nine-coordinate complexes with holodirected geometries, there are at present some examples in which hemidirected arrangements of ligands have been proposed. One of these examples, $[Pb(BzImH)_2py(H_2O)_2(NO_3)_2]\cdot(BzImH)_2py\cdot H_2O$ [15], has already been discussed in Section 2. Another is the complex $[PbL^1(NO_3)_2]$, which contains the oxo-aza-coronand 1,4,7,10-tetraoxa-13-azacyclopentadecane (L^1) [58].

In this complex, the lead(II) ion, which is not located above the centre of the macrocycle but is displaced toward the N(1), O(1) and O(4) atoms, interacts with all five of the donor centres of the coronand and with the four O atoms of two bidentate NO_3^- ions. All bond distances are below 3 Å and it was suggested that a SALEP might be located in the void that can be seen on the right-hand side of Fig. 32. This proposal is supported by the long bond distances near the void [namely Pb(1)–O(2), Pb(1)–O(3), Pb(1)–O(6) and Pb(1)–O(9), which are in the range 2.81–2.99 Å] and the short Pb(1)–N(1) bond distance [2.465(5) Å] *trans* to the pair.

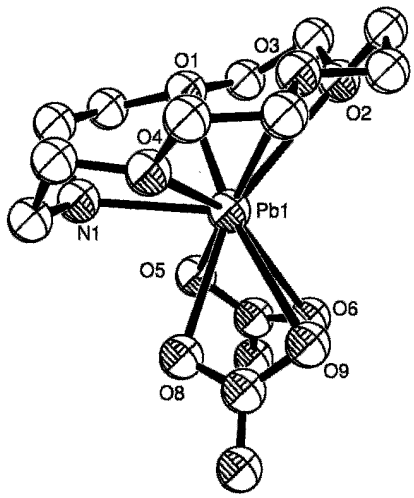


Figure 32. Structure of the complex $[PbL^1(NO_3)_2]$ (L^1 = oxo-aza-coronand, see text) (KUJCIB, [58]).

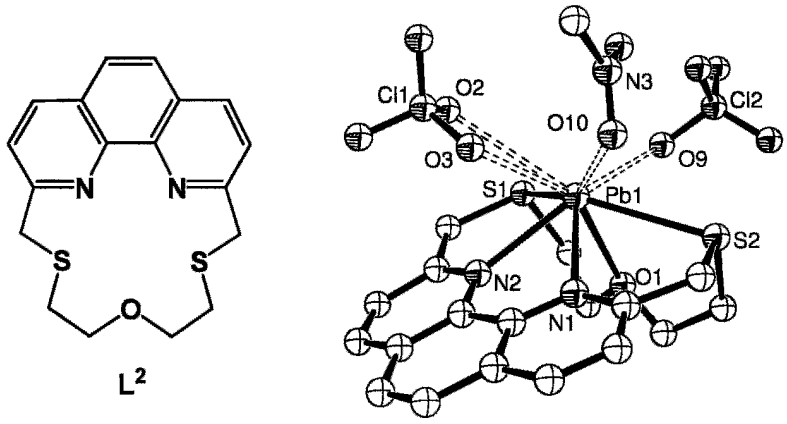
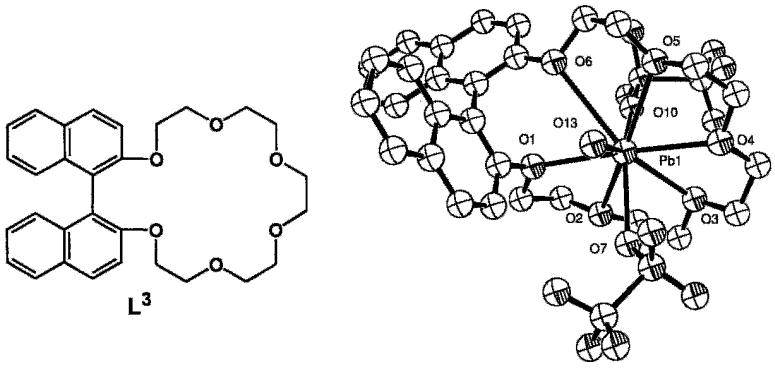


Figure 33. (a) The macrocycle 5-oxa-2,8-dithia[9]-(2,9)-1,10-phenanthrolinophane (L^2) and (b) the structure of its $[PbL^2(ClO_4)_2(MeNO_2)]$ complex (HUPCOK, [59]).

In the similar compound $[PbL^2(ClO_4)_2(MeNO_2)]$ {L^2 = 5-oxa-2,8-dithia[9]-(2,9)-1,10-phenanthrolinophane, see Fig. 33a}, the macrocycle also has five donor atoms, but there are two ClO_4^- anions instead of two nitrates. The very low basicity of the ClO_4^- anion is a possible reason that one of the perchlorate ions is partially displaced from the coordination sphere by a molecule of the solvent from which the complex was recrystallized. Consequently, the CN of nine is achieved once again, but the number of ligands increases and the void in the coordination sphere disappears. Nevertheless, it was suggested that a SALEP might be located in the coordination hemisphere left empty by the macrocyclic ligand [59].

A final interesting example of a nine-coordinated lead(II) complex is $[PbL^3(Otf)_2(H_2O)]$, which was recently prepared by Nagayama and Kobayashi [60] and contains a chiral crown ether ligand (L^3, see Fig. 34a). The lead(II) ion is coordinated by the six O atoms of the macrocycle [O(1) to O(6)], the two O atoms of the two triflate (trifluoromethanesulfonate, tfO^-) anions [O(7) and O(10)] and the O atom of a molecule of water [O(13)] (Fig. 34b).



(a) **(b)**

Figure 34. (a) The chiral crown ether L^3 and (b) the structure of its $[PbL^3(Otf)_2(H_2O)]$ complex (BEWHEQ, [60]).

The dihedral angle between the two naphthalene rings is responsible for the long Pb(1)–O(6) bond (3.109 Å) and the asymmetrical environment around the metal centre. This complex is a good catalyst for enantioselective aldol reactions in aqueous media. The high selectivity of the catalyst was explained by assuming that the water $H_2O(13)$ is replaced by one of the reagents under the reaction conditions [60].

There are more than thirty complexes identified by X-ray diffraction in which the lead(II) ion reaches *CN ten*. Many incorporate macrocycles as ligands and these mostly contain O and N as donor atoms, although some examples containing S donor atoms are also known [61].

A representative example of a ten-coordinated lead(II) complex is the helicate $[PbL^4](ClO_4)_2 \cdot 4MeCN$, where L^4 is a 34-membered macrocycle (see Fig. 35a) obtained by the Schiff-base condensation of α,α'-bis(2-aminophenoxy)-*o*-xylene and pyridine-2,6-dicarbaldehyde in the presence of lead(II) perchlorate trihydrate. When the solid obtained in this reaction was dissolved in acetonitrile, slow evaporation of the saturated solution yielded crystals that were suitable for study by X-ray diffraction [62].

The lead(II) ion is bound to all the potential donor atoms of the macrocycle (6N and 4O atoms), although the binding is stronger with the nitrogen donor atoms of each pyridine-2,6-diimine unit (average Pb–N bond distance 2.74 Å) than with the four phenoxy ether oxygens (average Pb–O bond distance 2.91 Å) (Fig. 35b).

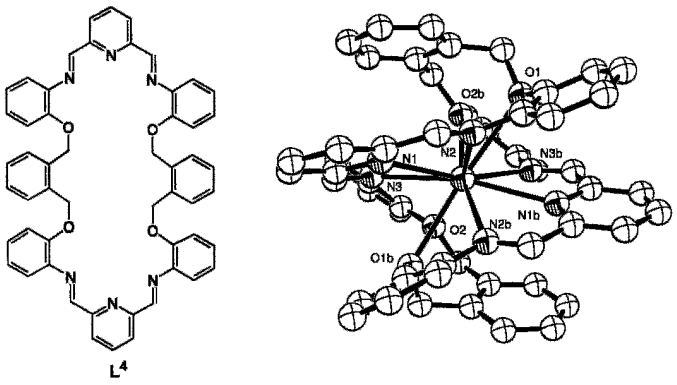
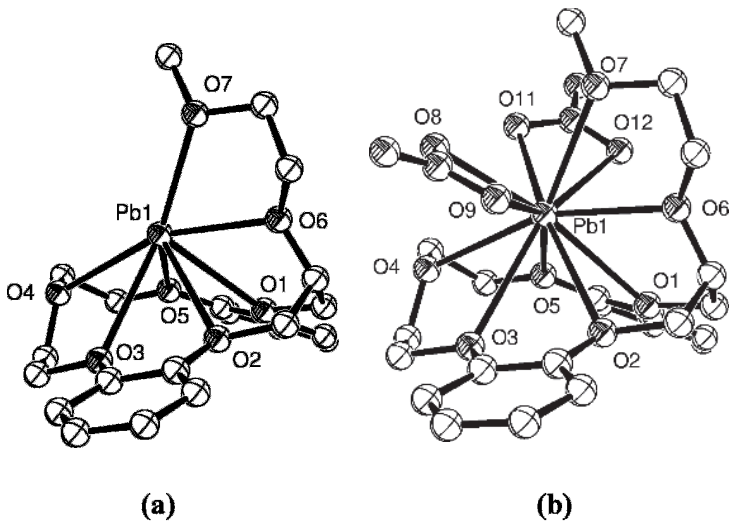


Figure 35. (a) The macrocycle L^4 and (b) the $[PbL^4]^{2+}$complex cation of the complex $[PbL^4](ClO_4)_2 \cdot 4MeCN$ (ZONWII10, [62]) showing the double helical structure and the Pb–N and Pb–O bonds. In order to better display the helical assembly of the cation, only nine metal-to-ligand bonds are shown [the Pb–O(2) bond is masked by the Pb–O(1b) bond].

The complex has a double helical structure derived from the twisting induced in the ligand by metal coordination. The space-filling diagram of the compound (not shown, see [61b]) indicates that the metal is fully encapsulated by the ligand and this rules out the presence of a SALEP.

According to FABMS experiments the lariat crown ether 2'-methoxyethyl-*sym*-dibenzo-16-crown-5 ether (L^5) shows preferential complexation with Pb(II) instead of other singly and doubly charged metal cations such as Zn(II), Hg(II), Ag(I) or Cu(II). In order to better understand this selective behaviour, the $[PbL^5(NO_3)_2]$ complex was isolated and studied by X-ray diffraction.

In the $\{PbL^5\}$ complex moiety (see Fig. 36a) the macrocycle adopts a twist-boat structure and the side-arm is positioned over the metal centre, thus enclosing the metal more tightly. The Pb(1)–O bond distances extend, as usual, over a broad range that in this case is between 2.634 Å for Pb(1)–O(6) and 3.013 Å for Pb(1)–O(3). If the latter value is considered as a significant bonding interaction (a reasonable hypothesis in a complex with such a high number of donor atoms in the coordination sphere), then all the lariat ether donor atoms are bound to the metal to give seven Pb(1)–O bonds. The coordination of lead(II) is completed by two bidentate nitrate anions, which occupy the space left by the lariat ether around the metal centre (see Fig. 36b). These four additional bonds are shorter than those formed by the ether and range between 2.580 Å for the Pb(1)–O(11) bond and 2.742 Å for the Pb(1)–O(12) bond. If we add all of the bonding interactions, the lead(II) ion has a nominal CN of *eleven*. This appears to be the only complex with this CN reported to date.



(a) **(b)**

Figure 36. Structure of the complex $[PbL^5(NO_3)_2]$ (TASCIZ, [62]) showing (a) the $\{PbL^5\}$ moiety and (b) the full coordination sphere including the two NO_3^- anions.

Complexes with *CN twelve* are also rather rare. An interesting lead(II) derivative with a $[PbO_{12}]$ kernel was prepared by Cramer et al. [63] by the reaction of lead(II) nitrate with the macrocycle obtained by heating thiamine (vitamin B_1) nitrate under reflux in absolute methanol [Eq. (5)]. This process induces thiazol displacement of one thiamine molecule, which is brought about by nucleophilic attack by the pyrimidine from a second thiamine, to give a cyclic hexameric 24-pyrimidinium crown-6 salt $[L(NO_3)_6]$ [63].

The reaction of $Pb(NO_3)_2$ with $[L(NO_3)_6]$ afforded the complex $L[Pb(NO_3)_6](NO_3)_2{\cdot}6H_2O$, which was studied by X-ray diffraction. This complex consists of 24-pyridinium crown-6 cations (L^{6+}) and $[Pb(NO_3)_6]^{4-}$ anions with additional NO_3^- anions to maintain its electric neutrality. These additional nitrates, although they do not occupy the centre of the macrocycle cavity, are closely associated with L^{6+}. The lead(II) $[Pb(NO_3)_6]^{4-}$ anionic complex has a close-to-cuboctahedral structure (Fig. 37) and the long Pb–O bond distances (2.906 and 2.744 Å) probably reflect the high coordination number of the metal in this complex. It is worth noting that, for a specific M–L bond, the higher CNs are usually associated with longer bond distances.

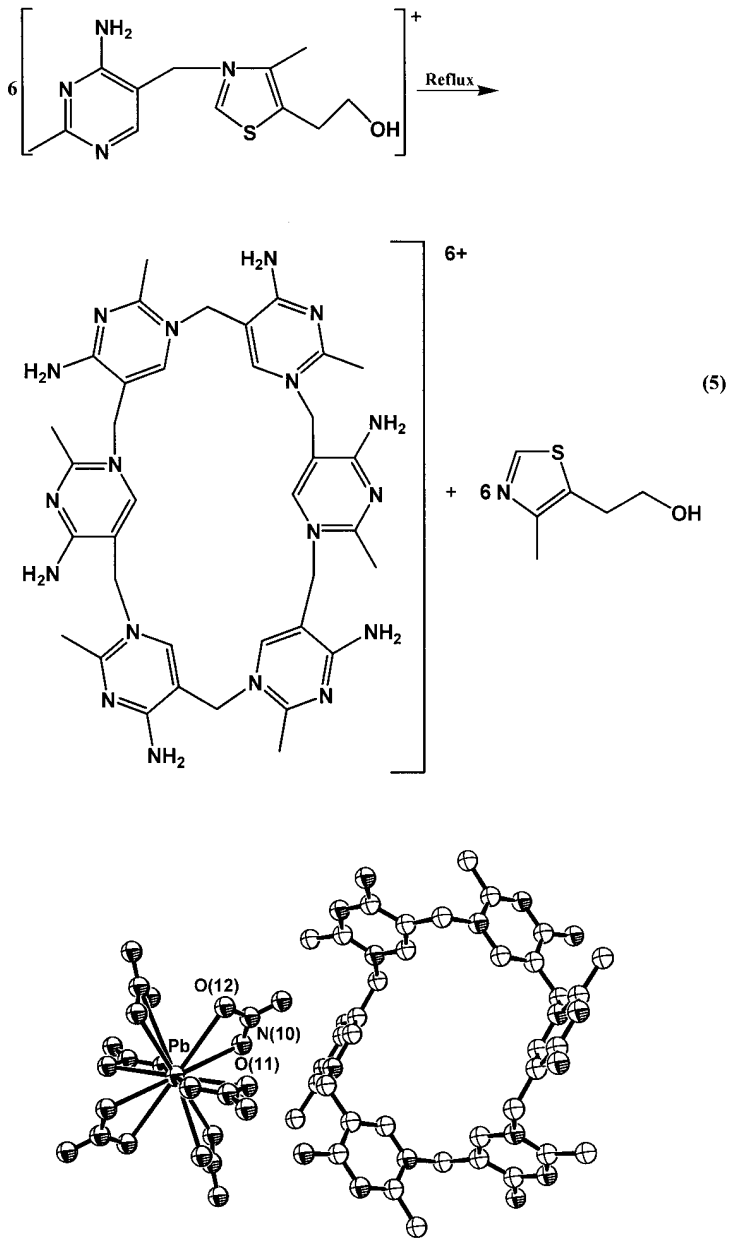


Figure 37. Structure of $L[Pb(NO_3)_6](NO_3)_2 \cdot 6H_2O$ (PILPIJ, [63]) showing the $[Pb(NO_3)_6]^{4-}$ anion with the lead(II) ion dodecacoordinated (left) and the macrocycle L^{6+} [right, see also Eq. (5)]. The non-coordinated nitrate counterions and the water molecules have been omitted for clarity.

3.6. Pb···Pb bonding interaction in lead(II) coordination compounds

The presence of metal-metal bonds in lead(II) organometallic compounds has been well documented (see, for example, diplumbenes in Chapter 3, Section 5) but on the other hand evidence of the formation of this type of bonding interaction in lead(II) coordination compounds is scarce.

Recently Rossi et al. [64] have analysed this topic in $[Pb^{II}(XS_2)_2]$ complexes (XS_2^- = S,S-bidentate monoanionic species, such as dithiocarbamates, xanthates, dithiophosphates, etc). These compounds often form supramolecular arrangements in the solid state that put the metal atoms at a short distance from each other.

One example is bis(pyrrolidinecarbodithioato)lead(II) (see Fig. 38a) that has a pyramidal coordination sphere, with the metal (or the SALEP) at the apex and the four S atoms making up the base. These units are stacked along the *c* axis placing each lead(II) centre at a distance from the sulphur atoms of the neighbouring unit (3.28 – 3.48 Å) that is shorter than the sum of the van der Waals radii (3.80 Å) and this suggests the presence of weak (secondary) interunit Pb-S bonds (Fig 38b).

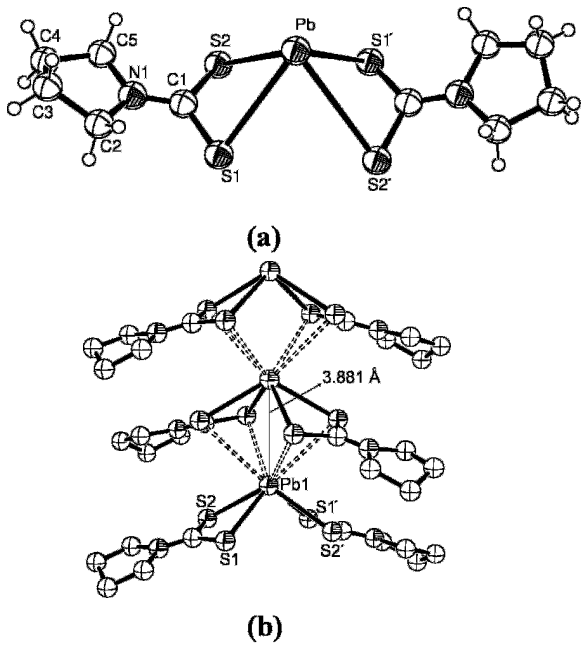
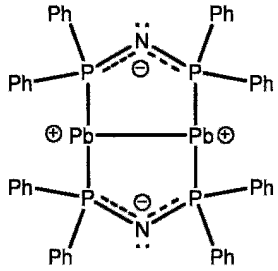


Figure 38. (a) The pyramidal unit of bis(pyrrolidinecarbodithioato)lead(II) [NINDUJ [64] and (b) packing of the units showing the short Pb···Pb distance.

If these secondary Pb-S interactions are taken into account, then the coordination number is eight, the polyhedron around the metal is a distorted square antiprism and the crystal structure is polimeric with successive antiprisms sharing the bases (Fig 38b) [64]. This arrangement places the metal centres at a distance [3.886(1) Å] that is shorter than the sum of the van der Waals radii (4.0, see Table 1) and not very far away from the upper limit observed in diplumbenes (2.903-3.527 Å) [65]. Furthermore, the SALEP of each Pb(II) ion is directed toward the next Pb(II) ion in the polymer and although a complete donation of the lone pair has been excluded, the presence of a weak metal-metal interaction via the SALEP cannot be discarded [64]. Note that this possibility would focus the pair along the Pb···Pb···Pb line, reducing its repulsion on the S atoms involved in the secondary Pb-S bonds. Nevertheless, it is likely that these last bonds are largely responsible for the proximity between the metal atoms.

A more obvious Pb-Pb bond occurs in the complex bis(μ_2-bis(diphenylphosphanyl)amido)-di-lead (see scheme 8) isolated from the reaction between lithium bis(diphenylphosphanyl)amide, $LiN(PPh_2)_2$ and $PbCl_2$ in THF [66]. The Pb-Pb distance [3.041(1) Å] and the large Pb,Pb coupling constant (7708 Hz) suggest the presence of a covalent Pb-Pb bond, so the compound is actually a lead(1+) complex.



Scheme 8

3.7. Coordination of lead(II) with biomolecules

Only a few lead(II) complexes with biomolecules have been structurally identified and these will be discussed below.

The complexes with citrate and D-gluconate have multimeric structures as well as a SALEP. The main structural characteristics of these compounds have recently been analysed by Godwin et al. and the interested reader is referred to this work [11].

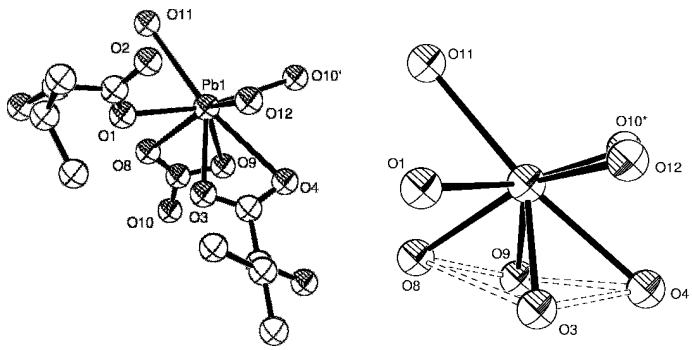
The structures of lead(II) complexes with some amino acids have been published since 2000. In their pioneering work, Gasque et al. [67a] studied the compound [Pb(Hasp)(NO_3)] (H_2asp = L-aspartic acid), a polymer with a six-coordinated metal centre. The arrangement of the coordination sphere of the complex, namely five oxygen atoms from the carboxylic acid groups belonging

to the aspartate ligand and one oxygen atom from the nitrate anion, leaves space for a SALEP [67a].

In the adduct $[PbBr_2(Hala)]$ (Hala = β-alanine zwitterion), the lead(II) ion is seven-coordinated and the coordination polyhedron is formed by four Br^- ligands, two O atoms from the bidentate carboxylic acid group of the Hala ligand and by one O atom from a neighbouring β-alanine zwitterion that chelates to another Pb(II) centre [67b]. The geometry of the coordination sphere can be described as a severely distorted pentagonal pyramid, with the main distortion arising from the irregular distribution of the equatorial bond angles. The bridging Br^- ions and Pb–O bonds, as well as the hydrogen bonds, which involve the $-NH_3^+$ group, give rise to a 3D structure.

A more comprehensive analysis of the interactions between lead(II) and amino acids was recently undertaken using mass spectrometry (ESI-MS) in 50% ethanolic solution [67c]. Under the experimental conditions used in this study, lead(II) has no preference for complexation of cysteine over other amino acids. This work also included the isolation of the complex $[Pb(val)_2(H_2O)_2(NO_3)_2]$ as well as the study of its structure by X-ray diffraction.

According to this analysis, the metal centre is eight-coordinated (see Fig. 39) via the oxygen donor atoms from a monodentate valine ligand [O(1)], from a second chelated valine ligand [O(3), O(4)], one chelated nitrate ligand [O(8), O(9)], another monodentate nitrate anion [O(10)*] and from two water molecules [O(11), O(12)]. The Pb(1)–O distances range between 2.36(1) [Pb(1)–O(3)] and 2.95(1) [Pb(1)–O(10)] Å. The arrangement of these donor atoms is rather irregular, as can be observed in Fig. 39b where the O(3), O(4), O(8), O(9) "plane" is highlighted in order to show the slight resemblance of the coordination sphere to a very distorted square antiprism. Although a holodirected geometry was proposed for the complex [67c], it is evident that a significant void exists in the upper part of Fig. 39b.



(a) **(b)**

Figure 39. (a) Structure and (b) kernel of the complex $[Pb(val)_2(H_2O)_2(NO_3)_2]$ (ESAPET, [67c]).

The reaction between lead perchlorate and glycylglutamic acid (H_2Gglu) was recently explored because this dipeptide is widely found in the calcium binding sites of EF-hand-proteins. The process was carried out in a 1:1 molar ratio and afforded the $[Pb(HGglu)(H_2O)](ClO_4)$ complex [68]. The structure of this compound (see Fig. 40) again shows the good affinity of lead(II) for the carboxylic acid group and the tendency of this type of lead(II) derivative to form polymeric arrangements.

Two $HGglu^-$ ligands chelate the metal centre and the Pb(1)–O bonds have distances between 2.451 and 2.734 Å. Another dipeptide ligand is only bound to the lead(II) ion through one of its O atoms [O(4)c*]. This last donor atom is also coordinated to two additional metal centres to form a 2D arrangement. The water molecule [O(6)], which completes the coordination up to six, also bridges two Pb(II) ions and extends the polymeric assembly to give a 3D structure. The $\{PbO_6\}$ kernel is irregular.

Lead is also very often used to solve the "phase problem" in studies into the crystal structures of proteins. Although the lead derivative used in these experiments usually contains Pb(IV), one would expect the solved crystal structures to give some clues concerning the bonding tendencies of the metal when it interacts with these types of macromolecules [11]. In the calcium binding proteins calmodulin and synaptotagmin, lead is located in coordination sites that contain carboxylate groups – as occurs in the complexes with amino acids described above. In synaptotagmin, lead is unable to reproduce the calcium binding mode and this characteristic might be responsible for the differences in the activity of the lead and calcium forms of the protein [11].

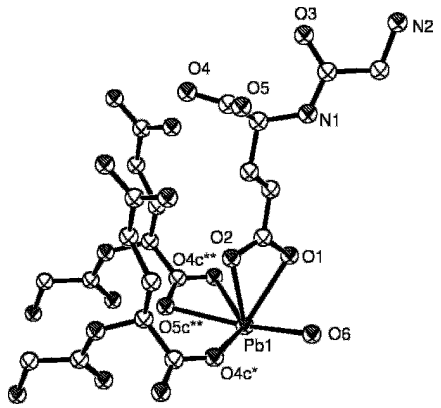


Figure 40. Structure of the complex $[Pb(HGglu)(H_2O)]ClO_4$ (OHETEA, [68]) (HGglu = monodeprotonated glycylglutamic acid).

As discussed above (see Section 2, Fig. 8 and refs. 22 and 23a), lead can occupy the catalytic site in the ALAD enzyme instead of zinc. In lead-ALAD, the metal is coordinated to three sulphur atoms of three cysteine (Cys) residues in a similar way as zinc in the wild-type ALAD. However, the SALEP on the lead centre reduces the capacity of the metal to interact with the substrate in the muted enzyme. According to a recent study involving X-ray absorption spectroscopy [23b], this coordination preference of lead(II) may encompass other cysteine-rich zinc proteins (transcription factors), even when additional sulphur atoms are available for coordination. Unlike the trigonal coordination of Zn(II) in ALAD, in these proteins the metal forms tetrahedral $\{ZnCys_4\}$ kernels, which means that introducing lead in order to give trigonal $\{PbCys_3\}$ kernels, probably results in significant differences in the folding properties of these transcription factors. This, in turn, could be related to the toxic effects of the lead compounds.

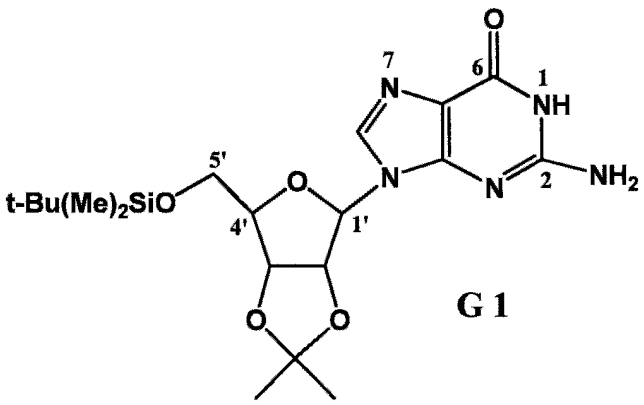
The structural information obtained in the solid state for the interaction between Pb(II) and nucleic bases, nucleosides, nucleotides and nucleic acids is rather scarce, although studies carried out in solution indicate that the primary binding sites for lead in a nucleic acid are probably the guanine and cytosine nucleic bases, followed by the phosphate unit [69]. In guanine and cytosine the expected donor centres are the N(7)/C(6)O and N(3)/C(2)O atoms, respectively, while in guanine or hypoxanthine residues, the metal is able to interact simultaneously with the nucleic base and the phosphate group to form macrochelates [69a]. It was also concluded that, in solution, the Pb(II) ion has the ability to interact strongly with two neighbouring phosphates [69b].

Although the available information regarding the interaction of lead(II) with these biochemical ligands in the solid state is rather scarce, the existing evidence is essentially consistent with the above statements. Thus, the Pb(II) ion interacts with the guanine nucleoside G **1** (5'-*tert*-butyl-dimethylsilyl-2',3'-*O*-isopropylidene guanosine, see Scheme 9) through the C(6)O group.

These types of compounds can self-assemble in a square co-planar array of four guanine bases, in which each base is hydrogen-bonded with its neighbours. This arrangement is termed G-quartet, guanine quartet or guanine tetrad (Fig. 41a).

In DNA, two or more G-quartets can stack on top of one another to form structures referred to as G-quadruplexes [70].

The extraction of Pb(II) picrate from water into $CDCl_3$ solution by the lipophilic G **1** and subsequent evaporation of the solvent gives a solid with the stroichiometry $[Pb(G\ \mathbf{1})_8](pic)_2$ [71]. When a single crystal of this solid was studied by X-ray diffraction, the results showed that the unit cell contains G-quadruplexes, each formed from two coaxial $[Pb(G\ \mathbf{1})_8]^{2+}$ octamers.



Scheme 9

In this octamer (see Fig. 41b), the metal centre coordinates eight oxygen atoms of the C(6)=O group to form a coordination sphere with a geometry intermediate between a cube and a square antiprism. The Pb–O bond distances are normal (2.66 ± 0.05 Å) and there is a clear structural similarity with the $[K(G\ \mathbf{1})_8]^+$ octamer, which gives rise to the possibility that the genotoxicity of lead could be derived from it replacing potassium in nucleic acid structures [71].

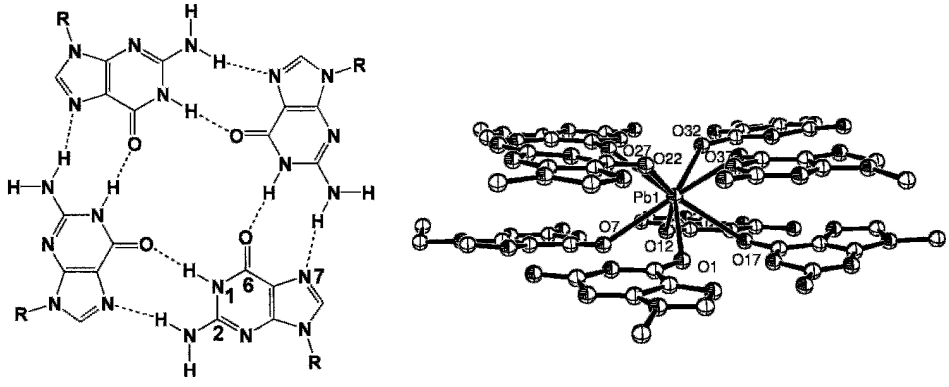
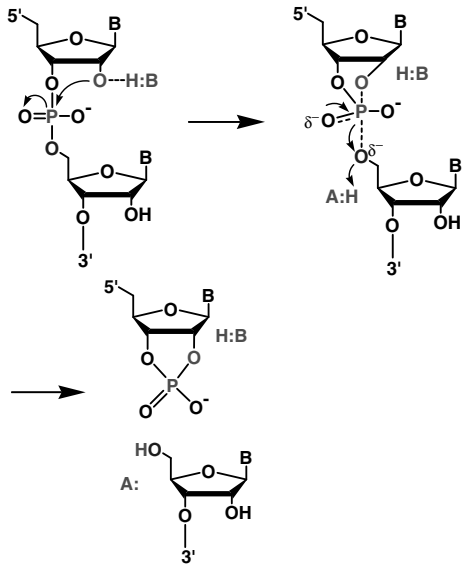


Figure 41. (a) G-quartet and (b) structure of the $[Pb(G\ \mathbf{1})_8]^{2+}$ cation in the $[Pb(G\ \mathbf{1})_8](pic)_2$ complex (G **1** = 5'-*tert*-butyl-dimethylsilyl-2',3'-*O*-isopropylidene guanosine, pic = picrate) (GUPWIX, [71]).

Some additional structural information regarding the interaction of Pb(II) with RNA was obtained from the study of leadzyme, a small ribozyme (RNA molecules with catalytic activity). These catalysts have been described as metalloenzymes because they usually require a divalent metal ion for full activity. The small rybozymes catalyse the cleavage of the phosphodiester backbone of RNAs, including their own phosphorus-oxygen bonds, according to the following mechanism (Scheme 10 adapted from [72]):



Scheme 10

The reaction is initiated by nucleophilic attack of a ribose 2'-hydroxyl group on the scissile phosphodiester bond. The reaction may require activation of the nucleophile by a general base B: and a protodonation to the leaving group by a general acid A:H [72].

In the case of leadzyme, the catalytic process needs the presence of the Pb(II) ion, which (in the form of the $[Pb(OH)]^+$ cation) possibly plays the role of both B: and A:H (see mechanism above).

The crystal structure of leadzyme was determined to a resolution of 1.8 Å (using $Sr^{2+}$to mimic Pb^{2+} with respect to binding) [73] and on the basis of the results it has been proposed that, in the pre-catalytic conformation of the enzyme, the metal located near the scissile bond binds directly to the nucleic bases (via N and O donor atoms) and to the phosphodiester backbone. Water molecules complete the coordination sphere, while the oxygen of the 2'-hydroxyl group involved in the nucleophilic attack on the cleavage-site is located 3.8 Å from the metal (see Fig. 42).

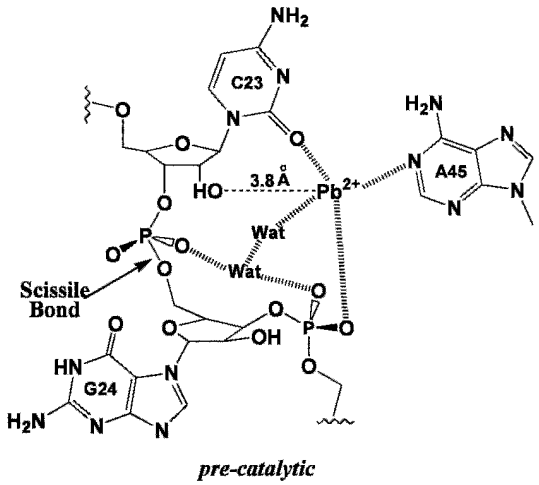
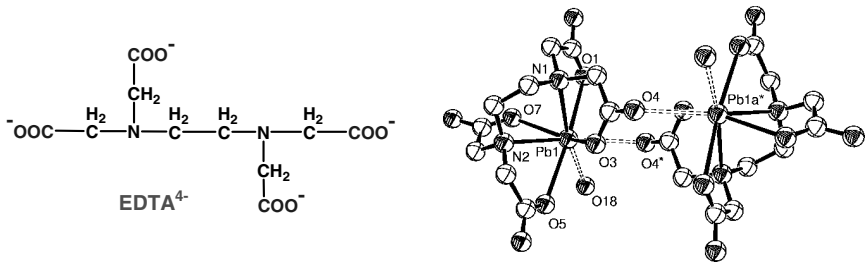


Figure 42. Proposed pre-catalytic arrangement in the Pb(II) site of the leadzyme (Adapted from Ref. 73).

3.8. Coordination of lead(II) to chelation therapy agents

There are several chelating agents used in conventional therapeutic treatments for lead poisoning (see Chapter 4, *7.1.1*). Perhaps the most commonly used system is the calcium disodium salt of ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid ($CaNa_2EDTA$) and this is probably the reason that the coordination chemistry of the Pb(II)/EDTA system has been thoroughly studied by X-ray diffraction [74]. Pb(II)/EDTA complexes can be divided into acidic (containing the H_2EDTA^{2-} anion) and neutral (containing the fully deprotonated acid, namely the $EDTA^{4-}$ anion; see Fig. 43a).

A representative example of these complexes is the acidic derivative $[Pb(H_2EDTA)(H_2O)]\cdot 0.5H_2O$. The crystal structure of this complex contains two crystallographically inequivalent lead(II) ions, Pb(1) and Pb(2) [75]. Each metal centre is coordinated to a hexadentate H_2EDTA^{2-} anion through two O atoms from the two deprotonated carboxylic acid groups, two O atoms from the protonated carboxylic acid groups and the two amine N atoms (see Fig. 43b). In the case of Pb(1), the Pb(1)–O bond distances range between 2.533 [O(3)] and 2.662 [O(1)] Å. The Pb(1)–N distances are 2.578 [N(1)] and 2.624 [N(2)] Å. This primary coordination sphere is complemented by two weaker Pb(1)–O bonds: one formed with the O(18) atom from the water molecule, which is located 2.930 Å from the metal centre, and another with the O atom from a carbonyl group belonging to a neighbouring complex [Pb(1)–O(4*) = 2.841 Å].



(a) **(b)**

Figure 43. (a) Fully deprotonated ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid; (b) partial structure of $[Pb(H_2EDTA)(H_2O)]\cdot 0.5H_2O$ (BIYLOK01, [75]) showing the molecule containing Pb(1), one of the two crystallographically inequivalent metal centres (see text).

The intermolecular interactions associate the complex into centrosymmetric dimers (see Fig. 43b). The molecule containing Pb(2) has a similar coordination environment, but the intermolecular Pb–O distance is significantly longer (3.196 Å) than in Pb(1) molecules – a situation that makes the dimeric arrangement rather dubious.

When the weak bonding interactions are not considered, the $\{PbO_4N_2\}$ coordination polyhedron can be described as a pentagonal bipyramid with an equatorial SALEP [74].

This mode of bonding and the stereochemical characteristics described above can be extended to the majority of the lead(II)/EDTA complexes that have been studied [74].

Another conventional chelating agent for lead(II) treatments is D-Penicillamine (DPA) (see Chapter 4, *7.1.2*), a sulfhydryl-containing amino acid (3,3-dimethyl-D-cysteine, see Fig. 44a) that is used for mild lead poisoning. The direct interaction between the Pb(II) ion and DPA was studied as early as 1974. The reaction between lead(II) nitrate and the chelating agent in water afforded crystals of the complex [Pb(DPA-2H)], which were studied by X-ray diffraction [76] (Fig. 44b). The results of this study showed that the dideprotonated amino acid (DPA-2H) binds strongly to the metal through the S atom from the ionised sulfhydryl group, one O atom belonging to the carboxylate group and the N atom from the amino group. These strong bonding interactions, together with a SALEP, conform to a tetrahedral geometry around the metal centre (Fig. 45a).

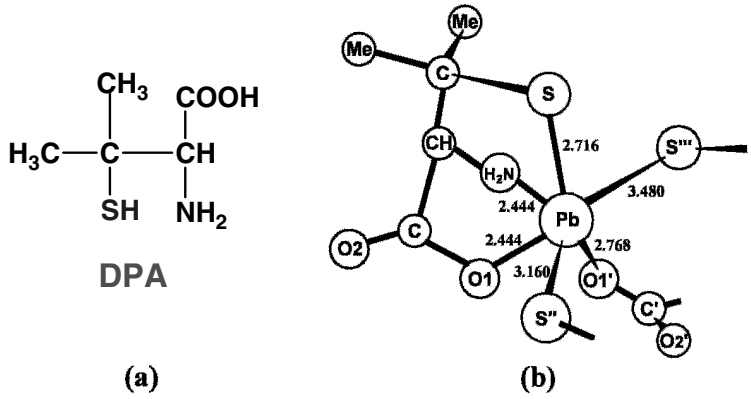


Figure 44. (a) D-Penicillamine (DPA); (b) structure of the [Pb(DPA-2H)] complex (Adapted from Ref. 76).

If the weaker intermolecular bonding interactions are taken into account, and if we also include all the potential donor atoms that are at a distance from lead less than the sum of the van der Waals radii, then the geometry of the coordination sphere becomes a distorted pentagonal bipyramid with the SALEP in an equatorial disposition (Fig. 45b).

Curiously, X-ray diffraction data are not available for the complex or complexes formed by meso-2,3-dimercaptosuccinic acid (DMSA), which is an effective oral chelator for lead(II) detoxification (see Chapter 4, *7.1.3*), even though the interaction of the disodium salt of meso-DMSA with lead nitrate was explored several years ago [77].

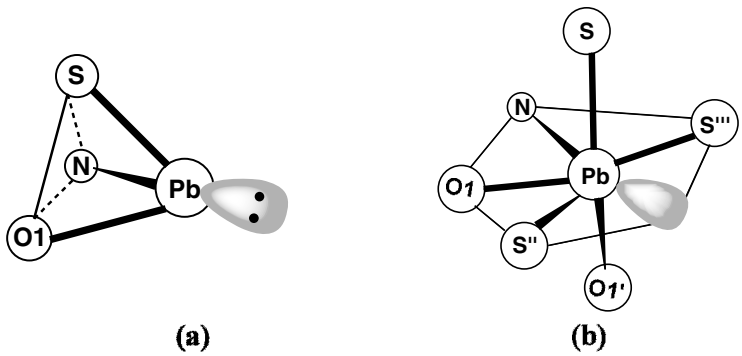
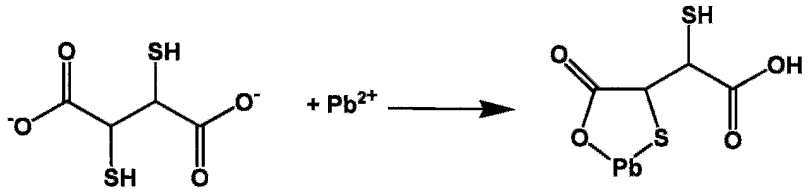


Figure 45. (a) Kernel of the complex [Pb(DPA-2H)] when only the strongest bonds are considered; (b) kernel including both the weak and strong bonding interactions [76].

According to IR measurements, the dianion of the acid is coordinated to the metal centre through one oxygen and one sulfur atom in the complex isolated in the solid state, [Pb(DMSA-2H)·2H_2O], (see scheme 11) [77].



Scheme 11

Bearing in mind the range of possibilities for deprotonation and the available donor centres in this ligand, it is clear that the Pb(II)/DMSA system warrants further exploration in the future.

Acknowledgment. Authors thank Prof. María V. Castaño, Prof. María S. García-Tasende and Dr. Ángeles Touceda for their help with the figures and schemes.

REFERENCES

[1] N.V. Sidgwick, The Chemical Elements and Their Compounds, Clarendon Press, Oxford, 1950.
[2] M. Kaupp and P.V.R. Schleyer, J. Am. Chem. Soc., 115 (1993) 1061.
[3] R.D. Hancock and A.E. Martell, J. Chem. Ed., 73 (1996) 654.
[4] S.S. Batsanov, J. Mol. Struct., 468 (1999) 151.
[5] P.G. Harrison, Coord. Chem. Rev., 20 (1976) 1.
[6] P.G. Harrison, Comprehensive Coordination Chemistry, G. Wilkinson, R.D. Gillard and J.A. McCleverty, (eds.), Vol. 3, Pergamon Press, Oxford, 1987.
[7] J. Parr, Polyhedron, 16 (1997) 551.
[8] J. Parr, Comprehensive Coordination Chemistry II, J.A. McCleverty, T.J. Meyer, G.F.R. Parkin, Eds., Vol. 3, Elsevier, Amsterdam, 2004.
[9] C.E. Holloway and M. Melnik, Main Group Met. Chem., 20 (1997) 399.
[10] L. Shimoni-Livny, J.P. Glusker and C.W. Bock, Inorg. Chem., 37 (1998) 1853.
[11] E.S. Claudio, H.A. Godwin and J.S. Magyar, Prog. Inorg. Chem., 51 (2003) 1.
[12] F.H. Allen, Acta Crystallogr., B58 (2002) 380.
[13] A. Walsh and G.W. Watson, J. Solid State Chem., 178 (2005) 1422.
[14] B.H. Hamilton, K.A. Kelly, T.A. Wangler, M.P. Espe and C.J. Ziegler, Inorg. Chem., 43 (2004) 50.
[15] S.V. Krivovichev, T. Armbruster and W. Depmeier, Mat. Res. Bul., 39 (2004) 1717.
[16] A. Morsali and A. Mahjoub, Inorg. Chem. Commun., 7 (2004) 915.
[17] R.D. Hancock, J.H. Reibenspies and H. Maumela, Inorg. Chem., 43 (2004) 2981.
[18] S. Ayyappan, G. Díaz de Delgado, A.K. Cheetham, G. Férey and C.N.R. Rao, J. Chem. Soc., Dalton Trans., (1999) 2905.

[19] (a) A.K. Hall, J.M. Harrowfield, A. Morsali, A.A. Soudi and A. Yanovsky, CrystEngComm, (2000) 13; (b) J. Harrowfield, Helv. Chim. Acta, 88 (2005) 2430.
[20] J.M. Harrowfiel, S. Maghaminia and A.A. Soudi, Inorg. Chem., 43 (2004) 1810.
[21] J.S. Casas, A. Castiñeiras, I. Haiduc, A. Sánchez, J. Sordo and E.M. Vázquez-López, Polyhedron, 13 (1994) 2873.
[22] E.K. Jaffe, Bioorg. Chem., 32 (2004) 316.
[23] B.M. Bridgewater and G. Parkin, J. Am. Chem. Soc., 122 (2000) 7140.
[24] J.S. Magyiar, T.C. Weng, C.M. Stern, D.F. Dye, B.W. Rous, J.C. Payne, B.M. Bridgewater, A. Mijovilovich, G. Parkin, J.M. Zaleski, J.E. Penner-Hahn and H.A. Godwin, J. Am. Chem. Soc., 127 (2005) 9495.
[25] S. Hino, M. Brynda, A.D. Phillips and P.P. Power, Angew. Chem. Int. Ed., 43 (2004) 2655.
[26] P.P. Power, J. Organometal. Chem., 689 (2004) 3904.
[27] T. Fjeldberg, H. Hope, M.F. Lappert, P.P. Power and A.J. Thorne, J. Chem. Soc., Chem. Commun., (1983) 639.
[28] K.W. Klinkhammer and W. Schwarz, Angew. Chem. Int. Ed. Engl., 34 (1995) 1334.
[29] B. Gehrhus, P.B. Hitchcock and M.F. Lappert, Angew. Chem. Int. Ed. Engl., 36 (1997) 2514.
[30] V.J. Catalano, B.L. Bennett and B.C. Noll, Chem. Commun., (2000) 1413.
[31] R. Usón, J. Forniés, L.R. Falvello, M.A. Usón and I. Usón, Inorg. Chem., 31 (1992) 3697.
[32] (a) A.C. Filippou, H. Rohde and G. Schnakenburg, Angew. Chem. Int. Ed., 43 (2004) 2243; (b) A.C. Filippou, N. Weidemann, G. Schnakenburg, H. Rohde and A.I. Philippopoulos, Angew. Chem. Int. Ed., 43 (2004) 6512.
[33] P.B. Hitchcock, H.A. Jasim, R.E. Kelly and M.F. Lappert, J. Chem. Soc., Chem. Commun., (1985) 1776.
[34] C.S. Weinert, I.A. Guzei, A.L. Rheingold and L.R. Sita, Organometallics, 17 (1998) 498.
[35] W.P. Leung, H.L. Hou, H. Cheng, Q.C. Yang, H.W. Li and T.C.W. Mak, J. Chem. Soc., Dalton Trans., (2003) 1505.
[36] L. Pu, B. Twamley and P.P. Power, Organometallics, 19 (2000) 2874.
[37] V.V. Krisyuk, I.A. Baidina and I.K. Igumenov, Main Group Met. Chem., 21 (1998) 199.
[38] M.J. Plater, S. Aiken, T. Gelbrich, M.B. Hursthouse and G. Bourhill, Polyhedron, 20 (2001) 3219.
[39] P. Pérez-Lourido, J. Romero, J.A. García-Vázquez, A. Sousa, Y. Zheng, J.R. Dilworth and J. Zubieta, J. Chem. Soc., Dalton Trans., (2000) 769.
[40] M. Afzaal, K. Ellwood, N.L. Pickett, P. O'Brien, J. Raftery and J. Waters, J. Mater. Chem., 14 (2004) 1310.
[41] A.N. Chekhlov, Russ. J. Coord. Chem., 26 (2000) 254.
[42] D.L. Reger, J.E. Collins, A.L. Rheingold, L.M. Liable-Sands and G.P.A. Yap, Inorg. Chem., 36 (1997) 345.
[43] (a) J.S. Casas, E.E. Castellano, J. Ellena, M.S. García-Tasende, A. Sánchez-Díaz, J. Sordo and M.J. Vidarte, P183, 7th FIGIPS, Lisboa (2003); (b) R. Pedrido, M.R. Bermejo, M.J. Romero, M. Vázquez, A.M. González-Noya, M. Maneiro, M.J. Rodríguez and M.I. Fernández, J. Chem. Soc., Dalton Trans., (2005) 572.
[44] M. Giselle and H. Fuess, Acta Cryst., C51 (1995) 242.
[45] D.B. Mitzi, J. Chem. Soc., Dalton Trans., (2001) 1.

[46] Y. Cui, J. Ren, G. Chen, W. Yu and Y. Qian, Acta Cryst., C56 (2000) e552.
[47] R.E. Marsh, Acta Cryst., B60 (2004) 252.
[48] A.E. Koziol, R.C. Palenik and G.J. Palenik, Inorg. Chim. Acta, 116 (1986) L51.
[49] M. Yamami, H. Furutachi, T. Yokoyama and H. Okawa, Inorg. Chem., 37 (1998) 6832.
[50] (a) D.L. Reger, M.F. Huff, A.L. Rheingold and B.S. Haggerty, J. Am. Chem. Soc., 114 (1992) 579; (b) D.L. Reger, J.E. Collins, A.L. Rheingold, L.M. Liable-Sands and G.P.A. Yap, Inorg. Chem., 36 (1997) 345.
[51] P. Bhattacharyya, J. Parr, A.T. Ross and A.Z. Slawin, J. Chem. Soc., Dalton Trans., (1998) 3149.
[52] M.R. St. J. Foreman, M.J. Plater and J.M.S. Skakle, J. Chem. Soc., Dalton Trans., (2001) 1897.
[53] J.D. Zubkowski, T. Hall, E.J. Valente, D.L. Perry, L.A. Feliu and J. Garmon, J. Chem. Crystall., 27 (1997) 251
[54] L.Tei, A.J. Blake, A. Bencini, B. Valtancoli, C. Wilson and M. Schröder, J. Chem. Soc., Dalton Trans., (2000) 4122.
[55] (a) D. Esteban, F. Avecilla, C. Platas-Iglesias, J. Mahía, A. De Blas and T. Rodríguez-Blas, Inorg. Chem., 41 (2002) 4337; (b) A.F. Danil de Namor, S. Chahine, D. Kowalska, E.E. Castellano and O. Piro, J. Am. Chem. Soc., 124 (2002) 12824; (c) E. Psillakis, J.C. Jeffery, J.A. McCleverty and M.D. Ward, Chem. Commun., (1997) 1965.
[56] K.L.V. Mann, J.C. Jeffery, J.A. McCleverty and M.D. Ward, J. Chem. Soc., Dalton Trans., (1998) 3029.
[57] (a) K.A. Beveridge and G.W. Bushnell, Can. J. Chem., 57 (1979) 2498; (b) J.M. Harrowfield, G.H. Shahverdizadeh and A.A. Soudi, Supram. Chem., 15 (2003) 367.
[58] K. Byriel, K.R. Dunster, L.R. Gahan, C.H.L. Kennard, J.L. Latten, I.L. Swann and P.A. Duckworth, Polyhedron, 11 (1992) 1205.
[59] M.C. Aragoni, M. Arca, F. Demartin, F.A. Devillanova, F. Isaia, A. Garau, V. Lippolis, F. Jalali, U. Papke, M. Shamsipur, L. Tei, A. Yari and G. Verani, Inorg. Chem., 41 (2002) 6623.
[60] S. Nagayama and S. Kobayashi, J. Am. Chem. Soc., 122 (2000) 11531.
[61] (a) R.W. Matthews, M. McPartlin and I.J. Scowen, Chem. Commun., (1996) 309; (b) D.E. Fenton, R.W. Matthews, M. McPartlin, B.P. Murphy, I. J. Scowen and P. A. Tasker, J. Chem. Soc., Dalton Trans., (1996) 3421.
[62] L. K. Liu, C.-S. Lin, D.-S. Young, W.-J. Shyu and C.-H. Ueng, Chem. Commun., (1996) 1255.
[63] (a) R.E. Cramer, K. A. Mitchell, A.Y. Hirazumi and S.L. Smith, J. Chem. Soc., Dalton Trans., (1994) 563; (b) R.E. Cramer, C.A. Waddling, C.H. Fujimoto, D.W. Smith and K. E. Kim, J. Chem. Soc., Dalton Trans., (1997) 1657.
[64] F. Caruso, M.L. Chan and M. Rossi, Inorg. Chem., 36 (1997) 3609.
[65] S. Hino, M. Olmstead, A.D. Phillips, R.J. Wright and P.P. Power, Inorg. Chem., 43 (2004) 7346.
[66] A. Winkler, W. Bauer, F.W. Heinemann, V. García-Moltalvo, M. Moll and J. Ellermann, Eur. J. Inorg. Chem., (1998) 437.
[67] (a) L. Gasque, S. Barnès, R. Ferrari, C.R. de Barbarín, M.J. Gutiérrez and G. Mandoza-Díaz, Polyhedron, 19 (2000) 649; (b) K. Reynolds, R.D. Willet and B. Twamley, Acta Crystallogr., E59 (2003) m402; (c) N. Burford, M.D. Eelman, W.G. LeBlanc, T.S. Cameron and K.N. Robertson, Chem. Commun., (2004) 332.

[68] R. Ferrari, S. Bernés, C.R. Barbarín, G. Mendoza-Díaz and L. Gasque, Inorg. Chim. Acta, 339 (2002) 193.
[69] (a) C.P. Da Costa and H. Sigel, Inorg. Chem., 39 (2000) 5985; (b) B. Knobloch, D. Suliga, A. Okruszek and R.K.O. Sigel, Chem. Eur. J., 11 (2005) 4163.
[70] T. Simonsson, Biol. Chem., 382 (2001) 621.
[71] F.W. Kotch, J.C. Fettinger and J.T. Davis, Org. Lett., 2 (2000) 3277.
[72] V.J. DeRose, Curr. Opin. Struc. Biol., 13 (2003) 317.
[73] J.E. Wedekind and D.B. McKay, Biochem., 42 (2003) 9554.
[74] R.L. Davidovich, Russ. J. Coord. Chem., 31 (2005) 455.
[75] A.L. Poznyak, G.N. Kupriyanova, I.F. Burshtein and A.B. Ilyukhin, Russ. J. Coord. Chem., 24 (1998) 825.
[76] H.C. Freeman, G.N. Stevens and I.F. Taylor Jr., J. Chem. Soc. Chem. Commun., (1974) 366.
[77] M. Rivera, H.V. Aposhian and Q. Fernado, J. Inorg. Biochem., 37 (1989) 283.

Lead - J.S. Casas and J. Sordo (Editors)

Chapter 3

Organolead chemistry

Ionel Haiduc[a], Hemant K. Sharma[b] and Keith H. Pannell[b]

[a]Facultatea de Chimie, Universitatea "Babes-Bolyai" Cluj-Napoca, RO-3400 Cluj-Napoca, Roumania

[b]Department of Chemistry, University of Texas at El Paso, El Paso, TX 79968, USA

1. INTRODUCTION

Organolead chemistry is dominated by the tetravalent state; however, recently fascinating chemistry of lead(II) organometallics has been developed. The interest for organolead compounds was stimulated by the discovery that their thermal decomposition generates free radicals [1] and by the large scale use of tetraethyllead as gasoline additive [2]. The chemistry of organolead compounds (as a whole or some of its particular aspects) is covered in several books [3], chapters in books [4], and journal reviews of various degrees of detail [5]. Some of these are largely outdated but still interesting and useful reading, as the basics of organolead chemistry have been long-established and are well covered in these sources. In this chapter the emphasis will be on recent results, but the early fundamental chemistry will not be ignored.

The lead-carbon bonds are weaker than the M-C bonds of other Group 14 metals; as a result organolead derivatives usually decompose at moderate temperatures (100-200°C), are easily cleaved by acids and other reagents and are readily oxidized. Quite often these properties are put to good use for synthetic purposes.

Lead forms homoleptic PbR_4 and PbR_2 compounds, lead-containing heterocycles, *e.g.* $R_2Pb(CH_2)_n$, and metallocenes $PbCp_2$ (where $Cp = \eta^5\text{-}C_5H_5$ and related groups). A large number of heteroleptic compounds of lead(IV), R_nPbX_{4-n} where X = H, halogen, OH, OR, SR, NRR' or other "anionic" groups are known. Mono-organolead derivatives, $RPbX_3$ (X = halogen or OOCR') are rare. Heteroleptic derivatives of lead(II), *i.e.* PbRX are also scarce. In addition, compounds containing Pb-Pb bonds and Pb-metal bonds have been characterized. Recent developments in organolead chemistry include the

discovery of diplumbylenes (formally $R_2Pb{=}PbR_2$) and cyclotriplumbanes, $[PbR_2]_3$, and observations of supramolecular self-assembly in several heteroleptic organolead derivatives.

2. TETRAORGANOLEAD COMPOUNDS, PbR_4

A large scale synthesis, used for the industrial preparation of lead tetraalkyls, PbR_4, R = Me or Et, which involves the reaction of lead-sodium alloy with alkyl chlorides, was discovered as early as 1853 [6], Eq. (1). Several catalysts have been used, *e.g.* aluminum chloride, organoaluminum compounds, Grignard reagents, etc.

$$4\ NaPb + 4\ RCl \rightarrow\ PbR_4\ + 3\ Pb\ + 4\ NaCl \qquad (1)$$

This reaction is practical only at industrial scale [7].

Lead metal can serve as starting material in a direct reaction with $Na[AlEt_4]$ and EtCl for the synthesis of $PbEt_4$ [8], and in some electrochemical syntheses with sacrificial lead electrodes, in the electrolysis of EtI with $Na[Et_3AlFAlEt_3]$ as electrolyte [9] or in the electrolysis of Grignard reagents in the presence of an alkyl halide (R = Me, Et) in the so-called NALCO process [10].

$$Pb + 2\ RMgX + 2\ RX \rightarrow PbR_4 + 2\ MgX_2 \qquad (2)$$

In laboratory scale preparations the reaction of $PbCl_2$ with organomagnesium [11], organolithium [12-14], or organoaluminum [15,16] reagents is preferred, Eqs. (3-5), although hexaorganodilead compounds can be formed from the same reagents under slightly modified conditions [12, 17].

$$2\ PbCl_2 + 4\ RMgX\ \rightarrow\ PbR_4 + Pb + 4\ MgClX \qquad (3)$$

$$2\ PbCl_2 + 4\ LiR \rightarrow\ PbR_4 + Pb + 4\ LiCl \qquad (4)$$

$$6\ PbCl_2 + 4\ AlR_3 \rightarrow\ 3\ PbR_4 + 3\ Pb + 4\ AlCl_3 \qquad (5)$$

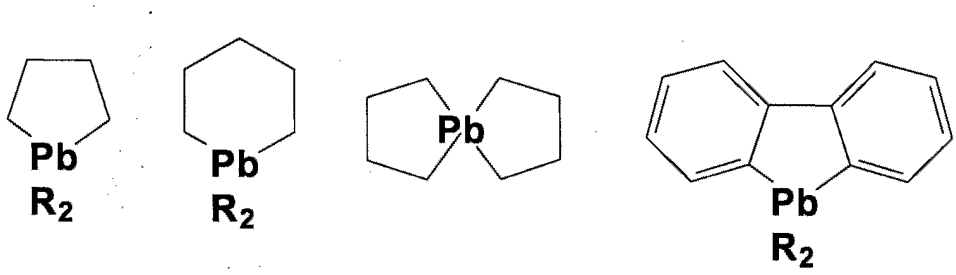
The incomplete use of lead in these reactions and the formation of a lead metal deposit can be avoided by carrying the reaction in the presence of excess alkyl halide, Eq. (6), M = Li or MgX [13, 18]:

$$PbCl_2 + 3\ MR + RX\ \rightarrow\ PbR_4 + 2\ MCl + MX \qquad (6)$$

With aluminum alkyls the addition of alkyl halides also prevents loss of lead metal [19], Eq. (7), R = Et.

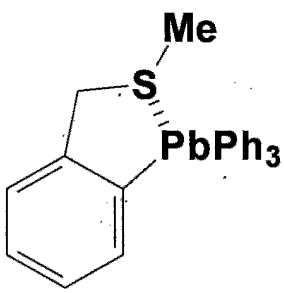
$$PbCl_2 + AlR_3 + RI \rightarrow PbR_4 + AlCl_2I \qquad (7)$$

Heterocyclic compounds (Scheme 1) with lead heteroatoms and four Pb-C bonds, *e.g.* saturated $R_2Pb(CH_2)_4$ and $R_2Pb(CH_2)_5$ [20] as well as the spirobicyclic derivative $(CH_2)_4Pb(CH_2)_4$ [21] have been prepared from di-Grignard reagents with R_2PbCl_2 and $PbCl_2$, respectively. Aromatic heterocycles $(C_6H_4)_2PbR_2$ have been synthesized with the aid of organolithium reagents [22].



Scheme 1

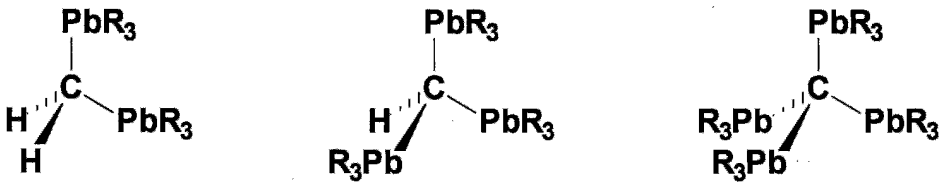
Tetraorganolead compounds are tetrahedral species, as demonstrated by several X-ray diffraction analyses of $PbMe_4$ (at 150K) [23], $PbPh_4$ [24], $Pb(C_6H_4Me\text{-}2)_4$ [25], $Pb(C_6H_4Me\text{-}3)_4$ [26], $Pb(C_6H_4Me\text{-}4)_4$ [27], $Pb(C_6F_5)_4$ [28], and others. In aromatic derivatives functional *ortho*-substituents able to interact with the metal distort the geometry. An example is the lead derivative of *ortho*-thioanisole, $(2\text{-}MeSCH_2C_6H_4)CH_2PbPh_3$, in which some Pb···S secondary interaction is suggested by the intramolecular interatomic distance of 3.953 Å, about 10% shorter than the Van der Waals distance [29] (Scheme 2).



Scheme 2

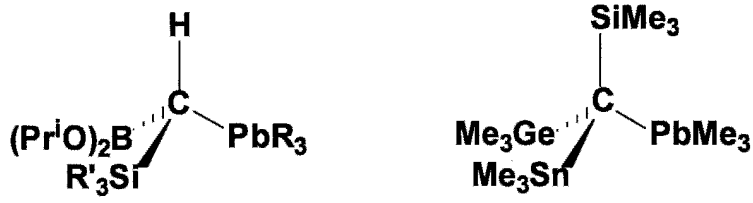
There are organolead compounds with four Pb-C bonds bearing functional groups in the organic substituents. Among these acyl plumbanes are of note. Contrary to the Si, Ge and Sn analogs acyl plumbanes, R_3Pb-COR, are very unstable and need kinetically stabilizing mesityl groups to be isolated and spectroscopically and structurally characterized [30]. Related $Ph_3PbC(O)OEt$ and $Ph_3PbC(O)NMe_2$ have also been reported but decompose rather easily [31]. Other functional derivatives will be mentioned below.

In a series of compounds containing four lead-carbon bonds the organolead moiety can be regarded as a substituent. The simplest examples are the partially or totally substituted triphenylplumbyl-methanes, *e.g.* $H_2C(PbPh_3)_2$, $HC(PbPh_3)_3$ and $C(PbPh_3)_4$, prepared from triphenylplumbyllithium with CH_nCl_{4-n} (n = 0 – 2) and $C(PbMe_3)_4$ prepared from trimethylplumbylsodium and CBr_4 [32] (Scheme 3).



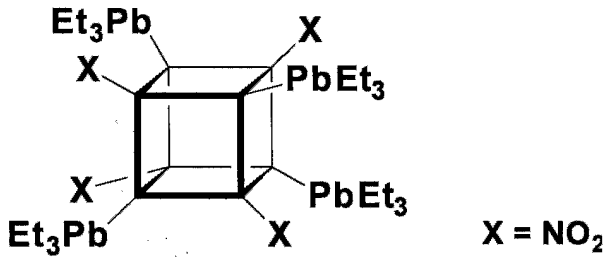
Scheme 3

Along these lines the substituted methane with four different substituents, one being a $PbPh_3$ group, is of note [33]. A further chemical novelty in this arena is the so-called catorcane molecule, $C(SiMe_3)(GeMe_3)(SnMe_3)(PbMe_3)$, in which all heavier group 14 elements are bonded to the central carbon atom [34] (Scheme 4).



Scheme 4

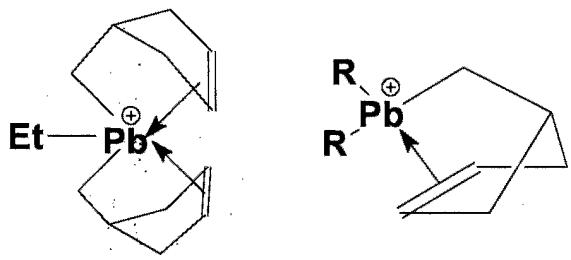
An interesting compound containing four triethyllead groups is a substituted nitrocubane derivative (Scheme 5), useful as intermediate for the synthesis of polynitrocubanes [35].



Scheme 5

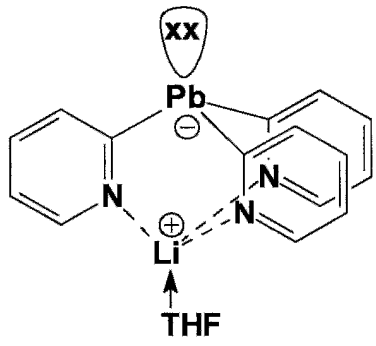
3. TRIORGANOLEAD SPECIES, $[Pb^{IV}R_3]^+$ and $[Pb^{II}R_3]^-$

Triorganolead(IV) cationic $[Pb^{IV}R_3]^+$ species are rare, but a plumbylium cation was reported as bis(η^1:η^2-cyclopentenylmethyl) (ethyl)lead$^+$, in which the positively charged lead atoms forms additional π-bonds with the C=C double bonds of the cyclopentenyl moieties [36]. Similar π-interactions of lead with the C=C double bond are observed in plumbanorbornyl cations, also containing positively charged lead [37] (Scheme 6).



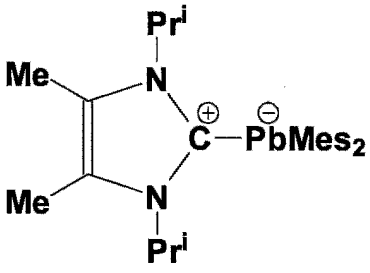
Scheme 6

A triorganolead(II) anion, $[PbR_3]^-$ in which R = 2-pyridyl, has been obtained as a lithium salt, $[Pb(2\text{-}Py)_3Li\cdot THF]$, from the reaction between $Pb(\eta^5\text{-}C_5H_5)_2$ with 2-pyridyllithium [38] (Scheme 7).



Scheme 7

A less common type of compound with three lead-carbon bonds include the plumbylene-carbene adducts formulated as zwitter-ionic [39] (Scheme 8).



Scheme 8

4. POLYNUCLEAR ORGANOLEAD(IV) COMPOUNDS CONTAINING Pb-Pb BONDS

The simplest are the hexaorganodileads, $R_3Pb\text{-}PbR_3$, of which the best known is the hexaphenyl derivative, formed under certain conditions in the reaction of $PbCl_2$ with PhMgBr, Eq. (8) [3b, 12, 17] or by Wurtz coupling of triorganolead halides with sodium in liquid ammonia {Eq. (9), R = Ph} [12, 40]. Hexaalkyl di-lead derivatives are also known, but they are only moderately stable; however, bulky organic groups provide kinetic stability and permit crystal structure determination [40].

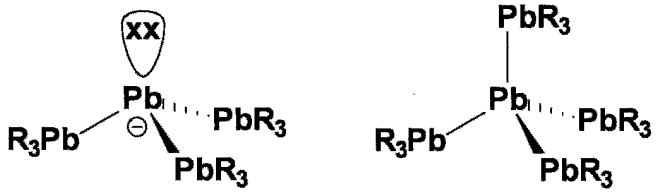
$$3\ PbCl_2 + 6\ RMgX \rightarrow R_3Pb\text{-}PbR_3 + Pb + 6\ RMgClX \qquad (8)$$

$$2\ R_3PbCl + 2\ Na \rightarrow R_3Pb\text{-}PbR_3 + 2\ NaCl \qquad (9)$$

The interatomic Pb-Pb distance in $Ph_3Pb\text{-}PbPh_3$ is 2.844(4) Å [41], but with the bulky $(Me_3Si)_3C$ (tsi) ligand, the stable hexaalkyldilead compound $(tsi)Me_2Pb\text{-}PbMe_2(tsi)$ has the longest Pb-Pb bond length recorded at 2.968(2) Å. The oligomeric tetra-lead anion $[Pb(PbPh_3)_3]^-$ (with a trigonal pyramidal structure, Pb-Pb distance 2.98 Å, Pb-Pb-Pb angle 93°) can be isolated at low temperature from the same reagents, Eq. (10) [42].

$$PbBr_2 + PhMgBr \xrightarrow{THF} [(MgBr(THF)_5]^+[Pb(PbPh_3)_3]^- \qquad (10)$$

A complex magnesium salt of the related tri[tris(biphenylylplumbyl)] plumbate anion, $[(THF)_3Mg(\mu\text{-}Cl)_3Mg(THF)_3][Pb(PbBp_3)_3]$ anion (Bp = biphenylyl, $-C_6H_4Ph$-4) was formed along with $Bp_3Pb\text{-}PbBp_3$ in the reaction of biphenylmagnesium chloride with $PbCl_2$ [43]. A well-defined branched penta-lead derivative, $Pb(PbPh_3)_4$, has been obtained by simultaneous oxidation and hydrolysis of Ph_3PbLi [12, 44]. The reaction of $PbCl_2$ with R_3PbLi also gives $Pb(PbR_3)_4$ [12] (Scheme 9).



Scheme 9

5. DIORGANOLEAD(II) COMPOUNDS, $:PbR_2$ (PLUMBYLENES), DIPLUMBENES, R_2PbPbR_2 AND CYCLOPLUMBANES $[PbR_2]_n$

The PbR_2 species can be isolated when stabilized by bulky, sterically demanding substituents; otherwise they are unstable and tend to disproportionate or to oligomerize. They are formed as intermediates in the synthesis of $PbAr_4$ and $Ar_3Pb\text{-}PbAr_3$ in the reactions of Grignard reagents with lead(II) halides (Eqs. 11-12) [3c, 17].

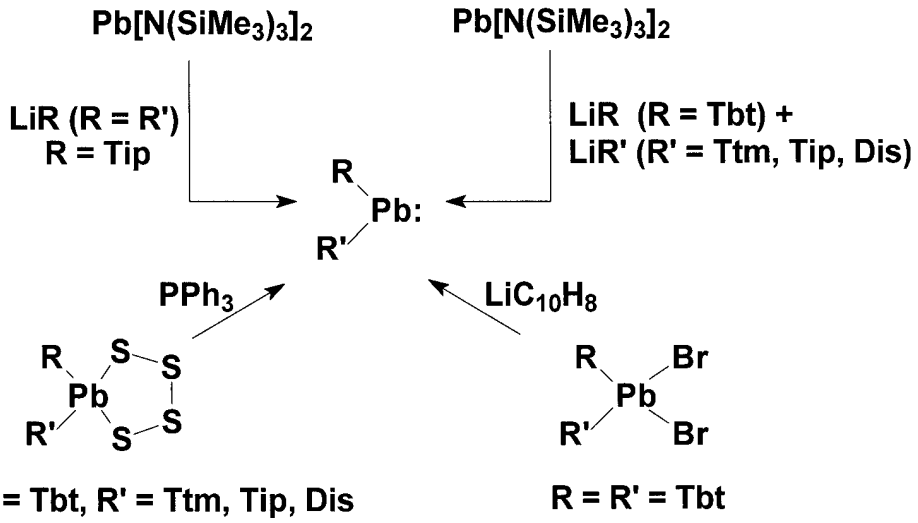
$$PbCl_2 + 2\ ArMgX \rightarrow PbAr_2 + 2\ MgClX \qquad (11)$$

$$3\ PbAr_2 \rightarrow Ar_3Pb\text{-}PbAr_3 + Pb \qquad (12)$$

Some stable diorganolead(II) compounds, *e.g.* $Pb[CH(SiMe_3)_2]_2$, could be isolated from the reaction of $PbCl_2$ with the corresponding organolithium reagent [45], Eq. (13).

$$PbCl_2 + 2\ LiCH(SiMe_3)_2 \rightarrow Pb[CH(SiMe_3)_2]_2 + 2\ LiCl \qquad (13)$$

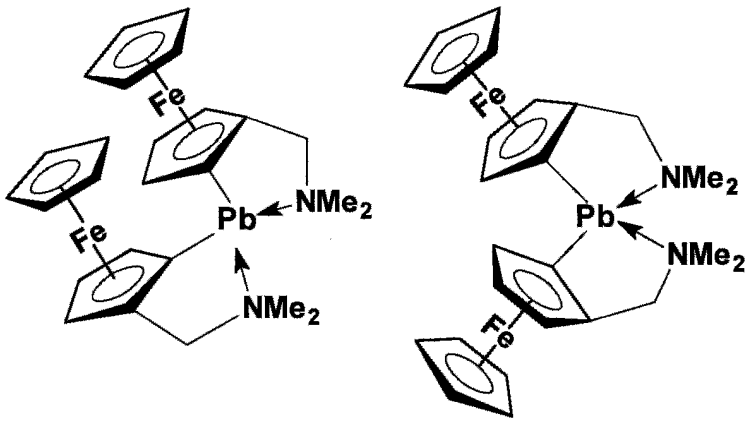
Other kinetically stabilized plumbylenes are $PbTip_2$ (Tip = 2,4,6-triisopropylphenyl) and PbRR' where R and R' are bulky substituents such as 2,4,6-tris[(bis(trimethylsilyl)methyl]phenyl, 2,4,6-tris[(trimethylsilyl)methyl]-phenyl] and bis(trimethylsilyl)methyl. These compounds are monomeric V-shaped molecules, as shown by X-ray diffraction [46]. Lead(II) silylamido derivatives can be used as starting materials for such plumbylenes, in reactions with organolithium reagents (Scheme 10) but debromination of $Tbt(R)PbBr_2$ with lithium-naphtalene $LiC_{10}H_8$ also yields plumbylenes, PbTbt(R) [46a].



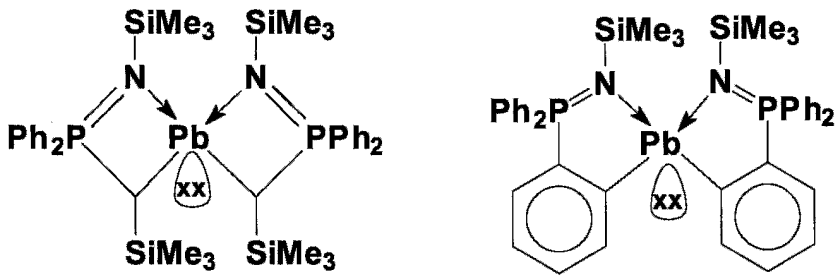
Tip = 2,4,6-tri(isopropyl)phenyl
Tbt = 2,4,6-tris[bis(trimethylsilyl)methyl]phenyl
Ttm = 2,4,6-tris[(trimethylsilyl)methyl]phenyl
Dis = bis(trimethylsilyl)methyl

Scheme 10

Plumbylenes can be stabilized by intramolecular donor-acceptor N→Pb bonds as shown for dimethylaminomethyl lead(II) derivatives of ferrocene (Scheme 11) [47] and for some aminophosphorane derivatives [48, 49] (Scheme 12).

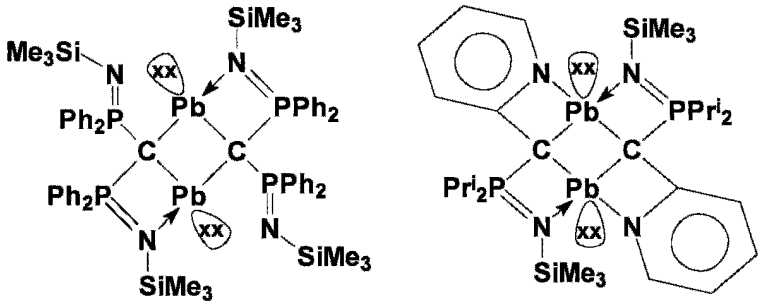


Scheme 11



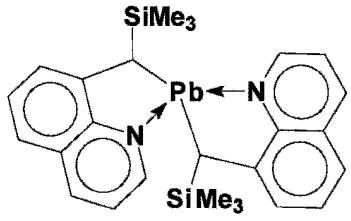
Scheme 12

Dinuclear plumbylenes with diplumbacyclobutane skeletons are also stabilized by appropriate iminophosphoranyl substituents capable of intramolecular N→Pb coordination, as shown in Scheme 13 [50].



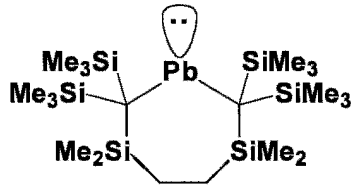
Scheme 13

8-Quinolinyl substituents have similar stabilizing effects through intramolecular coordination [51] (Scheme 14).



Scheme 14

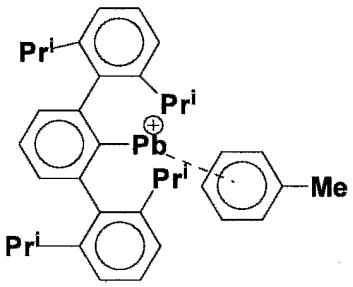
A seven-membered cyclic plumbylene bearing trimethylsilyl groups (Scheme 15) is protected against oligomerization by the bulky substituents. This was the first dialkyllead(II) compound structurally characterized by X-ray diffraction [52].



Scheme 15

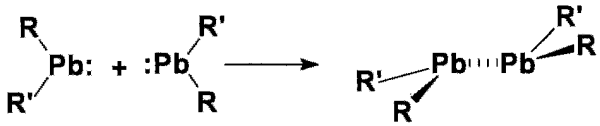
Demethylation of 2,6-$Trip_2C_6H_3PbMe$ (Trip = 2,4,6-$Pr^i_3C_6H_2$) with $B(C_6F_5)_3$ in toluene affords a quasi one-coordinate lead cation in the salt

$[2,6\text{-}Trip_2C_6H_3Pb(toluene)]^+[MeB(C_6F_5)_3]^-$ in which the metal is weakly solvated by toluene (Scheme 16 and Fig. 9, Chapter 2). DFT calculations on model compounds $[PhPb(C_6H_6)]^+$ and $[PhPb(C_6H_5CH_3)]^+$ were performed to analyze the interaction energies in such species [53].



Scheme 16

Plumbylenes can dimerize, as shown for some heteroleptic PbR'R" derivatives with R' = $SiMe_3$ and R" = 2,4,6-tris(trifluormethyl)phenyl or 2-*tert*-butyl-4,5,6-trimethylphenyl, to form diplumbenes with *trans*-bent structures (bending angle 40.8° and 46.5°, respectively) [54, 55] (Scheme 17).



Scheme 17

This dimerization is believed to occur through electron pair donor-acceptor interactions between two singlet plumbylenes (Fig. 1). The Pb-Pb distances of 3.370(1) Å and 3.537(1) Å are rather long compared to the calculated values of *ca.* 3.0 Å for $H_2Pb{=}PbH_2$ [56].

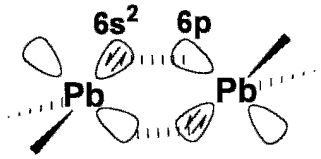
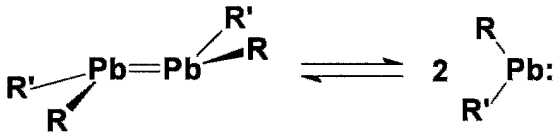


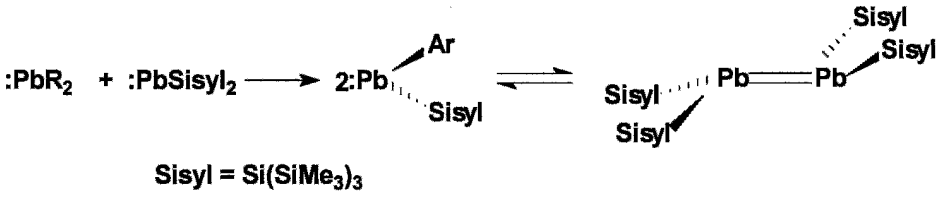
Figure 1. Dimerization of singlet plumbylenes

On the other hand, in the diplumbene obtained from $PbCl_2$ and TipMgBr [Tip = 2,4,6-tri(isopropyl)phenyl] the Pb-Pb bond is significantly shorter, 3.0515(3) Å, and bent angles of 43.9° and 51.2° are measured. This might be a true diplumbene, but it readily dissociates in solution to a monomeric plumbylene [57] (Scheme 18).



Scheme 18

Other diplumbenes with short Pb-Pb distances [2.903-2.9899(5) Å] are those containing $Si(SiMe_3)_3$ groups, which are formed by substituent redistribution [58] (Scheme 19).



Scheme 19

The cyclic oligomerization of plumbylenes is illustrated by the formation of a cyclotriplumbane in the reaction of $PbBr_2$ with 2,4,6-triethylphenyl magnesium bromide, Eq. (14) [5h, 59].

$$3\ PbBr_2 + 6\ RMgBr \rightarrow [3\ PbR_2] \rightarrow [PbR_2]_3 + 6\ MgBr_2 \qquad (14)$$

$$R = 2,4,6\text{-}Et_3C_6H_2$$

It is believed that the Pb_3 ring is not a classical homocycle, but rather a trimer of singlet plumbylene (Fig. 2) [5h, 59].

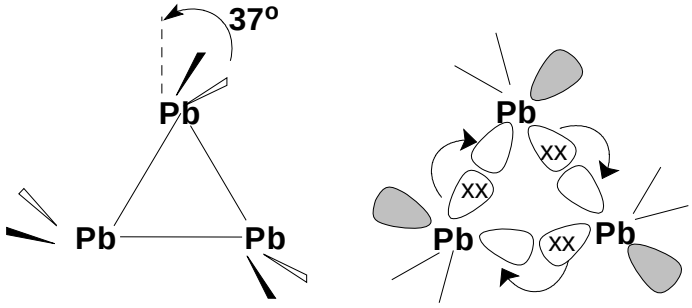
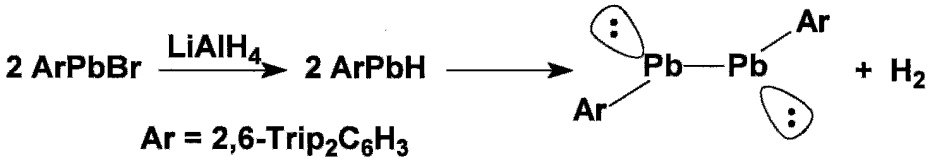


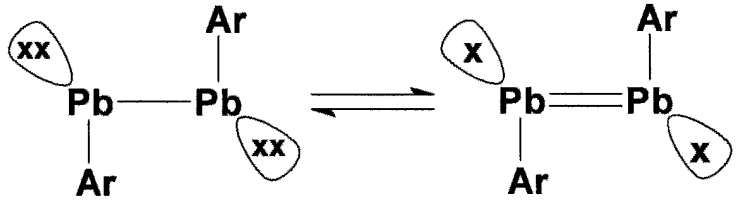
Figure 2. Trimerization of singlet plumbylenes

The reduction of RPbBr (R = 2,6-$Trip_2C_6H_3$) with $LiAlH_4$ yields a compound RPbPbR with a Pb-Pb interatomic distance of 3.1881(1) Å and a *trans*-bent geometry with a C-Pb-Pb angle of 90° [5h, 59] (Scheme 20).



Scheme 20

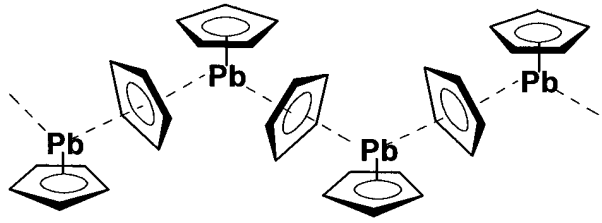
This geometry suggests that the lone pairs remain in the 6s orbitals of lead and the compound could be described as a diplumbene diradical rather than as a diplumbyne [60] (Scheme 21).



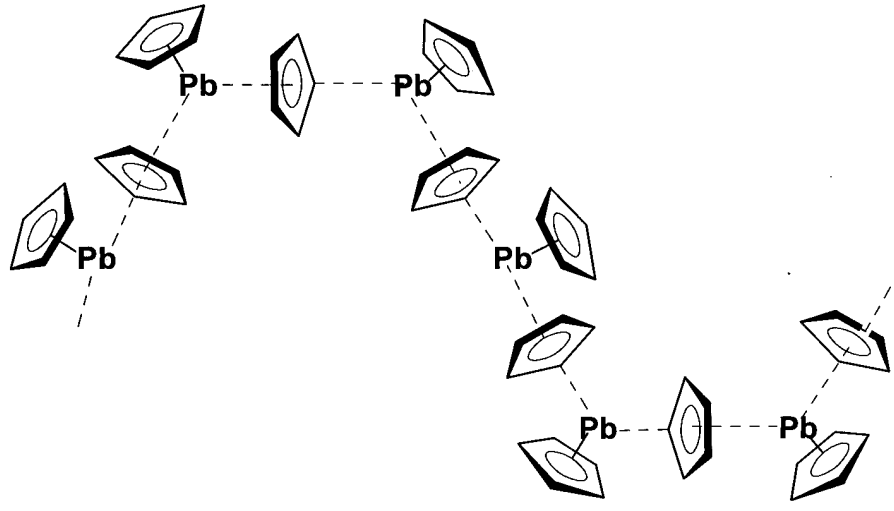
Scheme 21

6. PLUMBOCENES AND OTHER π-COMPLEXES

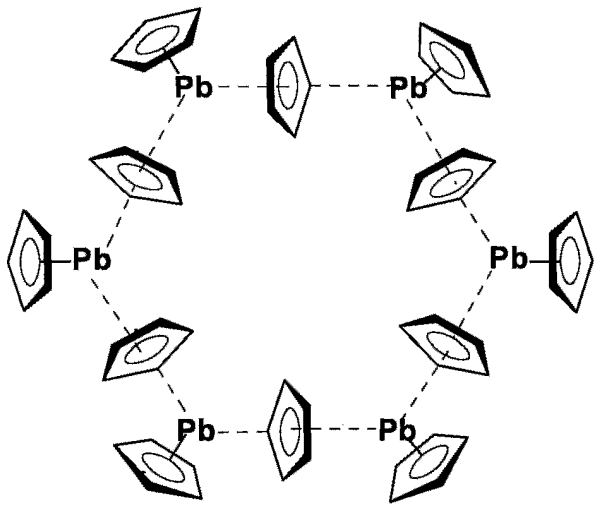
Bis(η^5cyclopentadienyl)lead(II) Pb(η^5-C_5H_5)$_2$, $PbCp_2$, ("plumbocene") was first prepared in 1956 from $PbCl_2$ and NaC_5H_5 [61]. Interestingly, this compound was found to display a unique polymorphism forming zig-zag chain [62], helical chain [63] and cyclic hexameric [63, 64] supramolecular self-assembled forms (Schemes 22-24). The compound is monomeric in the gas phase [65].



Scheme 22

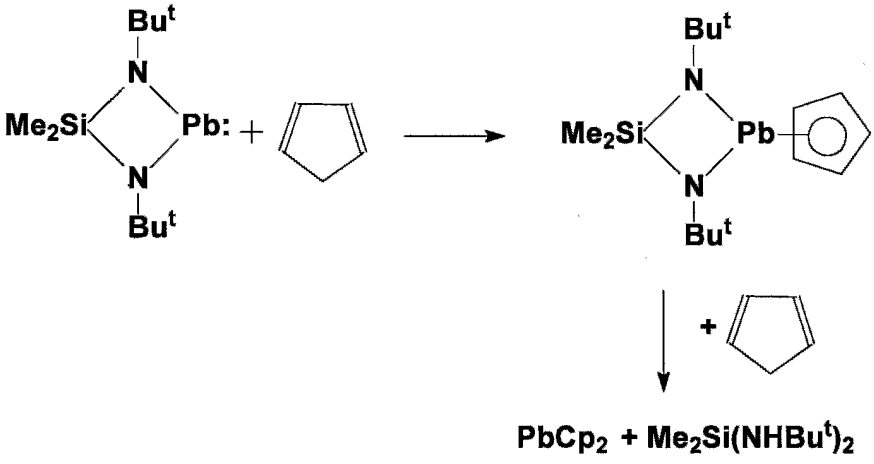


Scheme 23



Scheme 24

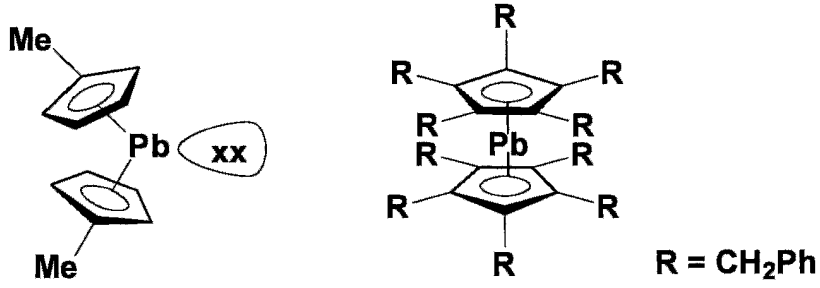
A surprising formation of plumbocene takes place in the reaction of a SiN_2Pb ring compound with cyclopentadiene; the monocyclopentadienyl intermediate was detected only spectroscopically [66] (Scheme 25).



Scheme 25

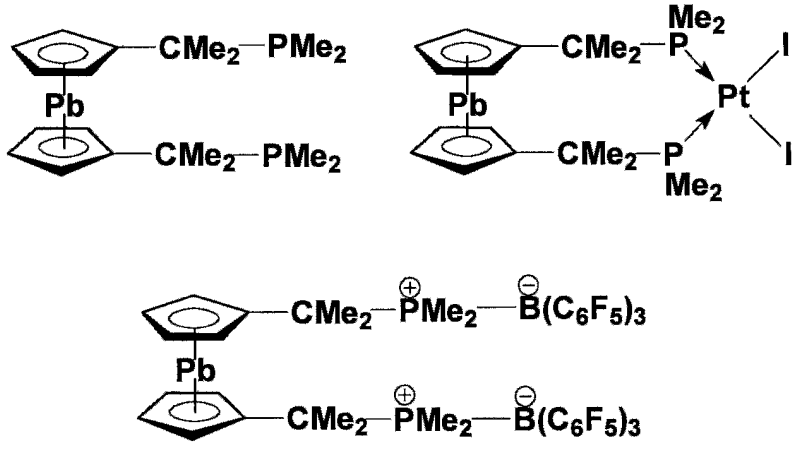
Unlike the unsubstituted plumbocene and methylsubstituted plumbocenes $Pb(\eta^5\text{-}C_5H_4Me)_2$ [67], and $Pb((\eta^5\text{-}C_5Me_4H)_2$ [68], which are bent metallocenes, the silylated derivative $Pb(\eta^5\text{-}C_5Me_4SiMe_2Bu^t)_2$ [69], the tris(isopropyl)

derivative $Pb(\eta^5\text{-}C_5H_2Pr^i_3\text{-}1,2,4)_2$ [70], and the pentabenzyl derivative $Pb[\eta^5\text{-}C_5(CH_2Ph)_5]_2$ [71] contain parallel cyclopentadienyl rings, obviously due to steric factors (Scheme 26). The metal-centered lone pair of electrons is stereochemically inactive in the later.



Scheme 26

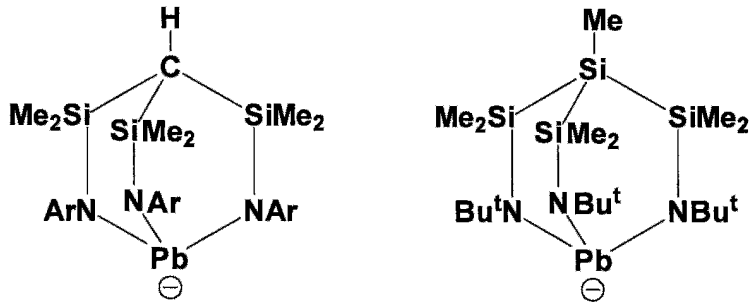
Novel plumbocene derivatives containing phosphinoalkyl ligands such as $Pb(\eta^5\text{-}C_5H_4CMe_2PMe_2)_2$ are also of note, due to their ability to form metal complexes, *e.g.* $[Pb(\eta^5\text{-}C_5H_4CMe_2PMe_2)_2PtI_2]$ and organoboron derivatives of the type $[Pb\{\eta^5\text{-}C_5H_4CMe_2PMe_2B(C_6F_5)_3\}_2]$ [72] (Scheme 27).



Scheme 27

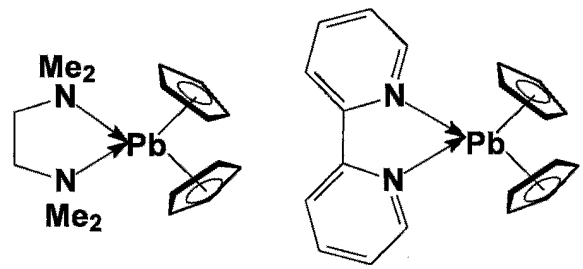
Plumbocene $Pb(\eta^5\text{-}C_5H_5)_2$ is a reactive compound. It exchanges cyclopentadienyl rings with $K[C_5Me_5]$ and $[Ca(C_5Me_5)_2](THF)_x$ to form

bis(pentamethyl-cyclopentadienyl) complexes, $Pb(\eta^5\text{-}C_5Me_5)_2$ [73]. The lability of plumbocene $Pb(\eta^5\text{-}C_5H_5)_2$ is illustrated by its reaction with lithiated primary amines, *e.g.* CyNHLi, leading to the formation of a cubane $Pb_4(NCy)_4$ formed by loss of the $\eta^5\text{-}C_5H_5$ ligands [74]. The reaction of plumbocene with lithiated tripodal amines also results in loss of cyclopentadienyl groups with formation of inorganic amidoplumbate anions shown in Scheme 28 [75].



Scheme 28

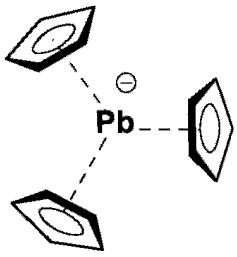
Plumbocene forms weakly bonded Lewis base adducts with bidentate nitrogen donors, such as tetramethylethylenediamine and 4,4'-Bipy, thus increasing the coordination number of lead and changing the molecular geometry (Scheme 29) [76]. Other adducts of $PbCp_2$ with tetracyanoethylene, tetracyanoquinodimethane and BF_3 have been reported [77].



Scheme 29

Plumbocene undergoes nucleophilic addition of C_5H_5 anions to form $[Pb(C_5H_5)_3]^-$ paddle-wheel anions (Scheme 30) [78, 79]. The syntheses and X-ray structures of such complexes, $[Na(PMDETA)]^+[(\eta^5\text{-}C_5H_5)_3Pb]^-$ and $[Mg(THF)_6][(\eta^3\text{-}C_5H_5)_3Pb]_2$ have been reported. The monomeric complex

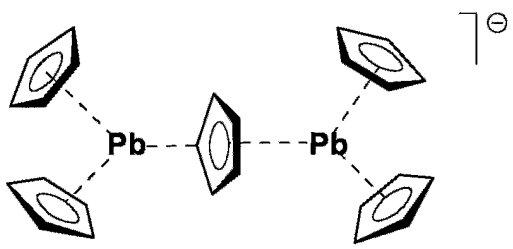
contains trigonal-planar $Pb(\eta^5\text{-}C_5H_5)_3$ units linked through bridging $\mu\text{-}\eta^5\text{-}C_5H_5$ ligands, to $[Na(PMDETA)]^+$ cations. *Ab initio* MO calculations show that the nature of these species is highly dependent on the alkali- or alkaline-earth-metal cation solvation. Thus, whereas unsolvated $[(\eta^5\text{-}C_5H_5)_2Pb(\eta^5\text{-}C_5H_5)Na]$ is a loose-contact complex of $Pb(C_5H_5)_2$ and NaC_5H_5, the $[Pb(\eta^5\text{-}C_5H_5)_3]^-$ species is best formulated as triorganometal anion [79].



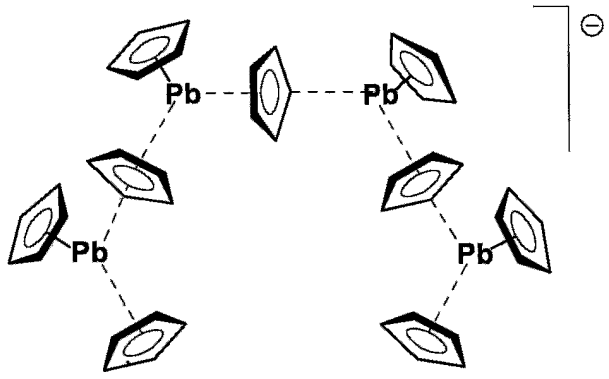
Scheme 30

The paddle-wheel tris(cyclopentadienyl)lead anion $[Pb(\eta^5\text{-}C_5H_5)_3]^-$ prepared from $\eta^5\text{-}C_5H_5PbCl$ and a potassium cyclopentadienyl derivative, KCp^{thf} (Cp^{thf} = 2-tetrahydrofurfurylcyclopentadienyl) in its potassium salt forms a honeycomb supramolecular structure *(vide infra)* [80]. The sodium salt $Na[(\eta^5\text{-}C_5H_5)_2PbCp^{thf}]\cdot 0.5THF$ also forms a two-dimensional supramolecular structure composed of $[Na(\eta^5\text{-}C_5H_5)_2PbCp^{thf}]_3$ rings [81].

Multideckker lead-cyclopentadienyl anions, *e.g.* $[Pb_2(C_5H_5)_5]^-$ and $[Pb_4Cp_9]^-$ are formed when the $C_5H_5^-$ anion reacts with plumbocene in the presence of crown ethers or cryptands, in addition to paddle wheel anions. Thus, $[Cp_2Pb(\eta^5\text{-}Cp)Na\cdot(15\text{-crown-}5)]$, $[K(2,2,2\text{-crypt})]^+[Cp_5Pb_2]^-\cdot THF$, $[Cp_2Pb(\mu\text{-}Cp)Pb(\eta^5\text{-}Cp)Cs(18\text{-crown-}6)]$ and the mixed salt $2[Li(12\text{-crown-}4)_2]^+[Pb_2Cp_5]^-$ $[Pb_4Cp_9]^-$ is formed from LiC_5H_5 and $Pb(C_5H_5)_2$ in the presence of the crown ether [82]. The structures of the anions are shown schematically in Schemes 31-32.

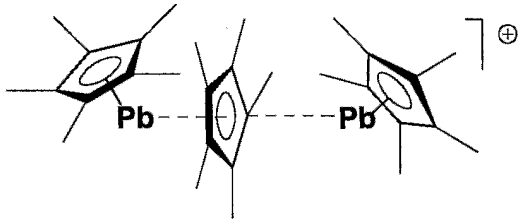


Scheme 31



Scheme 32

Triple-deckker cation salts $[Cp^*Pb(\mu,\eta^5)Cp^*PbCp^*]^+[B(C_6F_5)_4]^-$(where $Cp^* = C_5Me_5$) (Scheme 33) have been prepared by reacting $[PbCp^*]^+[B(C_6F_5)_4]^-$ with the plumbocene $PbCp^*_2$ [83].

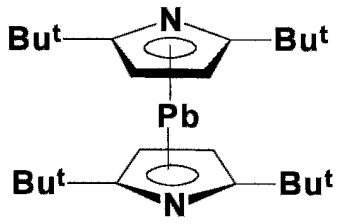


Scheme 33

A pentanuclear complex $[Pb_5(C_5Me_4SiMe_2Bu^t)_4(O_3SCF_3)_6]$ obtained from $Pb(\eta^5\text{-}C_5Me_4SiMe_2Bu^t)_2$ with CF_3SO_3H, has been described as a polyionic species formed by aggregation of four $[Pb(\eta^5\text{-}C_5Me_4SiMe_2Bu^t)]^+$ cationic species with the anion $[Pb(O_3SCF_3)_6]^{4-}$ on the basis of an X-ray diffraction analysis [84].

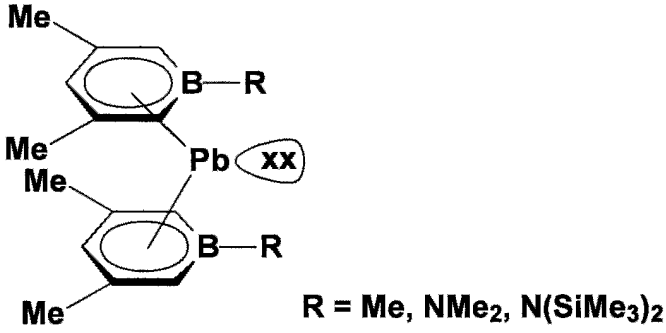
Bicylic hydrocarbons containing a fused cyclopentadienyl ring can also form lead(II) π-complexes and di[η^5-bis(trimethylsilyl)indenyl] $Pb[\eta^5\text{-}C_9H_5(SiMe_3)_3]_2$ has been reported [85].

Some π-complexes of lead derived heterocycles can be prepared. Pyrroles can form diazaplumbocenes and a *tert*-butyl substituted derivative (Scheme 34) has been structurally characterized [86].



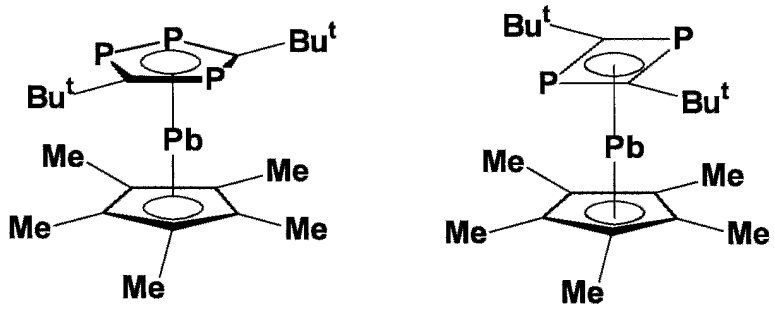
Scheme 34

Bis(1-methyl-boratabenzene)lead(II), $Pb(C_5H_5BMe)_2$ was obtained from LiC_5H_5BMe with $PbCl_2$ and investigated by X-ray diffraction. The compound is monomeric with a bent sandwich structure and a bending angle of 135.2(3)°. It forms 1:1 adducts with TMEDA and Bipy, which maintains the bending of the two π ligands; the 2,2-Bipy ligand is located in the pseudoequatorial plane [87]. Related $Pb(\eta^6\text{-}3,5\text{-}Me_2C_5H_3BNMe_2)_2$ and $Pb[\eta^6\text{-}3,5\text{-}Me_2C_5H_3BN(SiMe_3)_2]_2$ were obtained from $PbCl_2$ and lithium boratabenzenes. The compounds are monomeric, bent sandwich complexes [88] (Scheme 35).



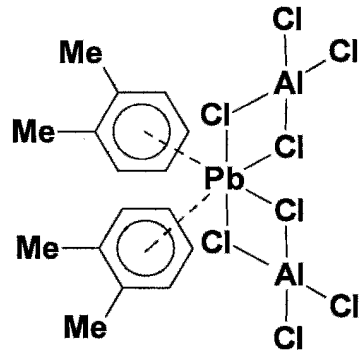
Scheme 35

Phospholes can also act as π-ligands (Scheme 36). $Bis(\eta^5\text{-}2,5\text{-di-}tert\text{-}butylphospholyl)lead$, $Pb(\eta^5\text{-}2,5\text{-}Bu^t_2C_4P)_2$, is one such complex [89]. A η^5-3,5-di(*tert*-butyl)-1,2,4-triphospholyl mixed ligand complex also containing η^5-pentamethylcyclopentadienyl ligands, $Cp^*Pb(\eta^5\text{-}Bu^t_2C_2P_3)$ has been crystallographically confirmed [90]. A unique 1,3-diphospha-cyclobutadienyl complex $Cp^*Pb(\eta^4\text{-}P_2C_2Bu^t_2)$ (Scheme 36), prepared from PbI_2 with $Cp_2Zr(PCBu^t)_2$, was described as a 24-electron nido-5-vertex cluster [91].



Scheme 36

Lead can also form π complexes with arenes as η^6-ligands. Thus, the compound $[Pb(\eta^6\text{-}C_6H_4Me_2\text{-}1,2)_2][AlCl_4]_2$ with a bent sandwich structure has been obtained simply from $PbCl_2$, $AlCl_3$ and 1,2-xylene, and structurally characterized by X-ray diffraction. The tetrachloroaluminate anions are interacting with the lead atom to complete its coordination environment (Scheme 37) [92]. A benzene complex, $[(\eta^6\text{-}C_6H_6)Pb(AlCl_4)]\cdot C_6H_6$ has also been described [93].



Scheme 37

The $[PbX_3]^+$ (X = H, Me, Cl) cation aromatic bonding to toluene, with a η^6-connection was the subject of *ab initio* calculations (binding energies, geometric structures and charge distribution), in a comparative study with similar Si, Ge and Sn derivatives [94].

7. ORGANOLEAD HYDRIDES

Because of the weakness of the Pb-H bonds, the organolead hydrides are thermally very unstable compounds and can be prepared only at negative temperatures (usually below -50°C). They are obtained by reducing organolead halides with $LiAlH_4$ in ethereal solvents [95] or with KBH_4 in liquid ammonia [96]. The organolead hydrides are also light- and air-sensitive.

An interesting property of organolead hydrides is their ability to undergo addition to unsaturated compounds (hydroplumbation) [97] which may turn out to become useful.

8. ORGANOLEAD HALIDES

8.1. Organolead(IV) halides

The preparation methods of organolead halides are now classical. They may differ with the nature of the halogen. Organolead(IV) chlorides and bromides, sometimes iodides, can be prepared by strictly controlled reactions of tetraalkyl with halogens (to avoid complete dealkylation) at low temperatures (for the synthesis of triorgano- and diorganolead halides) {Eqs. (15-16)} [98] or by cleavage of hexaalkyl (or aryl) dilead compounds (to give trialkyllead halides) {Eq. (17)} [99], and by reactions of tetraalkyllead compounds with gaseous HCl (or HBr) in suitable solvents (to produce either R_3PbCl or R_2PbCl_2, excellent for aromatic compounds) {Eqs. (18-20)} [100]. Other halogenation reagents can be used, *e.g.* thionyl chloride for the synthesis of R_3PbCl or Ph_2PbCl_2 (R = alkyl) from the corresponding tetraorganolead compounds {Eq. (21)} [101]. The method of choice is the chlorination with ammonium hexachloroplumbate(IV) due to high yields and more convenient handling of a solid chlorinating agent, Eqs. (22-23) [102].

$$PbR_4 + X_2 \rightarrow R_3PbX + RX \quad (15)$$

$$PbR_4 + 2\ X_2 \rightarrow R_2PbX_2 + 2\ RX \quad (16)$$

$$R_3PbPbR_3 + X_2 \rightarrow 2\ R_3PbX \quad (17)$$

$$PbR_4 + HCl \rightarrow R_3PbCl + RH \quad (18)$$

$$PbR_4 + 2\ HCl \rightarrow R_2PbCl_2 + 2\ RH \quad (19)$$

$$R_3PbPbR_3 + 3\ HCl \rightarrow R_3PbCl + PbCl_2 + 3\ RH \quad (20)$$

$$PbR_4 + SOCl_2 \rightarrow R_3PbCl + RSOCl \quad (21)$$

$$PbR_4 + [NH_4]_2[PbCl_6] \rightarrow R_3PbCl + PbCl_2 + RCl + 2\ NH_4Cl \quad (22)$$

$$R_3PbPbR_3 + [NH_4]_2[PbCl_6] \rightarrow 2\ R_3PbCl + PbCl_2 + 2\ NH_4Cl \quad (23)$$

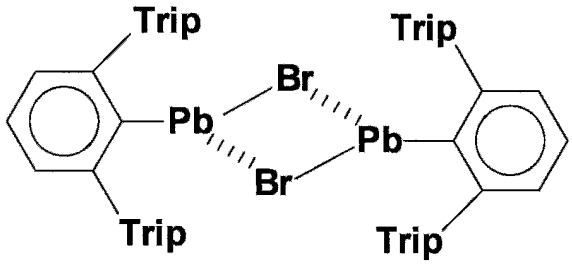
Organolead(IV) fluorides are best prepared from organolead hydroxides or acetates with alcoholic solutions of HF or by metathesis of aryllead bromides or iodides with potassium fluoride [103]. Aryllead trifluorides are obtained from "arylplumbonic acids" RPb(O)OH, with HF [104].

Alkyllead triiodides are prepared by oxidative addition of alkyl iodides to PbI_2, catalyzed by $SbMe_3$ [105]. Diorganolead(IV) diiodides are difficult to obtain because they disproportionate with formation of organolead triiodides; these are also unstable and decompose into lead(II) iodide with elimination of alkyl iodides [106].

The monoorganolead(IV) trihalides are all unstable. Theoretical *ab initio* pseudopotential computations show that tetravalent lead compounds are destabilized by electronegative substituents, and this explains their instability [107].

8.2. Organolead(II) halides

Organolead(II) halides, RPbX, are relatively new organolead compounds and can be made only with bulky organic groups which provide kinetic stability. Thus, the reaction of $LiC_6H_3Trip_2$-2,6 with $PbBr_2$ in diethyl ether gave a lead(II) compound associated as a dimer through bromine bridges, [2,6-$Trip_2C_6H_3Pb(\mu$-$Br)]_2$, formed through secondary interactions [108] (Scheme 38).



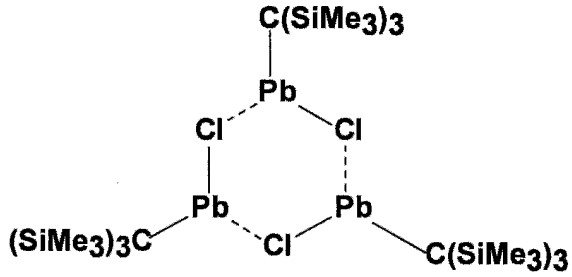
Scheme 38

The compound reacts with Grignard reagents to give new plumbylenes, 2,6-$Trip_2C_6H_3PbR$ (R = Me, Bu^t, Ph) and with $W(CO)_5THF$ to form a plumbylyne complex, $[(CO)_4W]_2(\mu\text{-}PbC_6H_3Trip_2\text{-}2,6)(\mu\text{-}Br)$ [108] (see Fig. 12, Chapter 2). With $Na[CpM(CO)_3]$ (M = Cr, Mo, W) the divalent bromide gives $Cp(CO)_3M\text{-}PbC_6H_3Tip_2\text{-}2,6$ with bent M-Pb-C bond angles [108.6(2)-113.58(9)°] and long Pb-M bonds [Pb-Cr 2.9092(9) Å, Pb-Mo 2.9845(7) Å; Pb-W 2.9809(10) and 3.0055(6) Å] suggesting single Pb-M bonding [109].

The lead(II) compound $[2,6\text{-}Trip_2C_6H_3Pb(\mu\text{-}Br)]_2$ reacts with *cis*-$[Mo(N_2)_2(PMe_3)_4]$ to form *trans*-$[2,6\text{-}Trip_2C_6H_3Pb{\equiv}MoBr(PMe_3)_4]$ which contains a lead-molybdenum triple bond [2.5495(8) Å] and a practically linear Mo-Pb-C fragment [177.8(2)°, see Fig. 12, Chapter 2] [110], and with *cis*-$[W(N_2)(PMe_3)_4]$ to give a similar compound containing a Pb≡W triple bond, *i.e.* *trans*-$[2,6\text{-}Trip_2C_6H_3Pb{\equiv}WBr(PMe_3)_4]$ [111].

The reaction of $PbBr_2$ with $Li(C_6H_3Trip_2\text{-}2,6)$ and $Et_2O{\cdot}LiC_6H_3\text{-}2,6\text{-}(C_6H_2\text{-}2,6\text{-}Pr^i_2\text{-}4\text{-}Bu^t)_2$ gave similar $[ArylPb(\mu\text{-}Br)]_2$ dimers with very bulky substituents and some of their interesting chemistry, like formation of new plumbylenes and diplumbenes, has been presented [112].

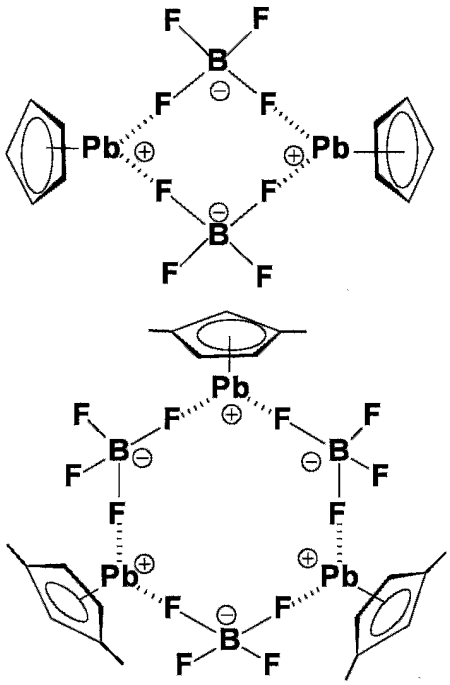
The tris(trimethylsilyl)methyllead(II) chloride is a cyclic trimer, $[(Me_3Si)_3CPbCl]_3$, (Scheme 39) containing a Pb_3Cl_3 ring of boat conformation (Pb-Cl distances in the range 2.71-2.76 Å) [113], and a cyclic dimer $[Me_3SiNPhC(SiMe_3)_2CPbCl]_2$ with alternating short Pb-Cl (2.609 Å) and long Pb···Cl secondary bonds (3.276 Å) has also been reported [114].



Scheme 39

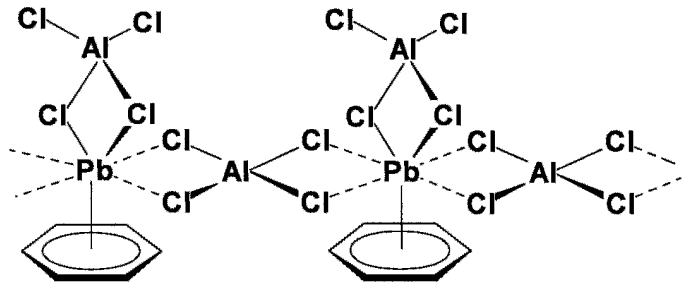
Monocyclopentadienyllead(II) complex halides belong to the same class. Halides CpPbX (X = Cl, Br, I) and acetate, CpPb(OAc), were prepared by reacting plumbocene with HX and the iodide CpPbI was also obtained from $PbCp_2$ with iodine or methyl iodide. It was suggested that the compounds are polymeric (*i.e.* supramolecular) with bridging X groups [115]. Cyclopentadienyllead(II) tetrafluroborate, $CpPb[BF_4]$ is a dimer with

tetrafluoroborato bridges leading to an eight-membered ring [116], and (1,3-di-*tert*-butylcyclopentadienyl) lead(II) tetrafluoroborate is a cyclic trimer with the same type of bridges [117] (Scheme 40).



Scheme 40

An η^6-arene complex cation coordinating $[AlCl_4]^-$ anions both as chelating and bridging groups is the benzene derivative η^6-$C_6H_6Pb[AlCl_4]_2 \cdot C_6H_6$ which forms supramolecular chains [118] (Scheme 41).



Scheme 41

9. ORGANOLEAD COMPOUNDS CONTAINING Pb-O, Pb-S, Pb-Se, Pb-Te BONDS

9.1. Oxygen compounds

Organolead compounds containing Pb-O bonds include hydroxides, alkoxides, carboxylates, and derivatives of other oxo acids. A few of these have been structurally characterized. Organolead oxides $R_3PbOPbR_3$ and R_2PbO have been mentioned in the literature, but no structural characterization is available. Triorganolead hydroxides, R_3PbOH, are obtained by alkaline hydrolysis of the chlorides [119]. The aromatic derivative Ph_3PbOH is obtained by wet oxidation of $Ph_3PbPbPh_3$ with potassium permanganate [120]. Dihydroxo compounds $R_2Pb(OH)_2$ are probably the immediate products of hydrolysis of dichlorides, but they dehydrate easily to ill-defined oxides, R_2PbO, of unknown structure [121]. Another class of organolead-oxygen compounds is represented by the so-called plumbonic acids RPb(O)(OH) of unknown structure (certainly polymeric), obtained by hydrolysis of monoorganolead carboxylates [122, 123].

Organolead carboxylates are important compounds. Triorganolead derivatives, $R_3PbOOCR'$, and diorganolead dicarboxylates, $R_2Pb(OOCR')_2$, are prepared by cleavage of organic groups from tetrasubstituted PbR_4, with carboxylic acids, under controlled conditions, Eqs. (24-25). Numerous examples are known [124].

$$PbR_4 + R'COOH \rightarrow R_3PbOOCR' + RH \quad (24)$$

$$PbR_4 + 2\ R'COOH \rightarrow R_2Pb(OOCR')_2 + 2\ RH \quad (25)$$

Mixed aryl-methyllead(IV) trifluoroacetates, $ArMePb(OOCCF_3)_2$, can be obtained by reacting aryllead tris(trifluoroacetates), $ArPb(OOCCF_3)_3$ with tetramethylsilane and trifluoroacetic acid [124b].

Monoorganolead carboxylates, $RPb(OOCR)_3$, are limited to aryllead derivatives, $ArPb(OOCR)_3$. Such compounds can be prepared from diaryllead carboxylates with mercury(II) carboxylates, Eq. (26) [125], by reaction of lead tetracarboxylates with mercury diaryls, Eq. (27) [126], or by redistribution between lead(IV) acetate and diphenyllead diacetate, Eq. (28), or tetraphenyllead, Eq. (29) [127].

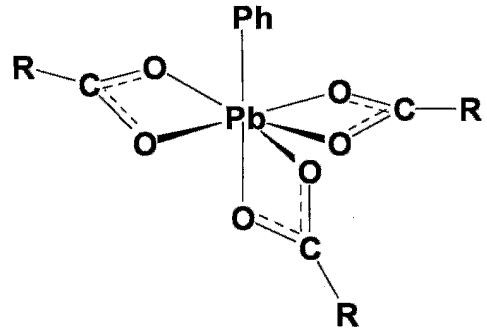
$$Ar_2Pb(OOCR)_2 + Hg(OOCR)_2 \rightarrow ArPb(OOCR)_3 + ArHgOOCR \quad (26)$$

$$Pb(OOCR)_4 + HgAr_2 \rightarrow ArPb(OOCR)_3 + ArHgOOCR \quad (27)$$

$$Pb(OAc)_4 + Ph_2Pb(OAc)_2 \rightarrow 2\ PhPb(OAc)_3 \quad (28)$$

$$3\ Pb(OAc)_4 + PbPh_4 \rightarrow 4\ PhPb(OAc)_3 \qquad (29)$$

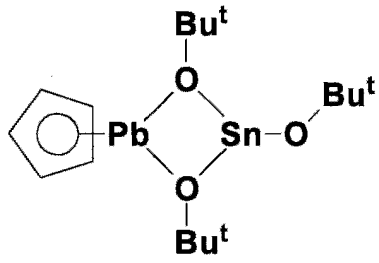
Monoaryllead(IV) carboxylates, *e.g.* $4\text{-}RC_6H_4Pb(OOCMe)_3$ (R = Me, Cl) contain seven-coordinate lead in a trigonal bipyramidal geometry with the phenyl group in axial position [128] (Scheme 42).



Scheme 42

Organolead(IV) alkoxides, $R_nPb(OR)_{4-n}$, obtained by metathesis of organolead chlorides with sodium alkoxides or by azeotropic dehydration of mixtures of $R_nPb(OH)_{4-n}$ with alcohols [129], are moisture sensitive compounds.

In the reaction of a lead(II) mixed alkoxide complex $[KPb(OBu^t)_3]_x$ with $\eta^5\text{-}C_5H_5SnCl$ a transfer of the cyclopentadienyl ligand to lead occurred, with the formation of an unusual lead-oxygen compound, $\eta^5\text{-}C_5H_5Pb(\mu\text{-}OBu^t)_2SnOBu^t$ [130] (Scheme 43).

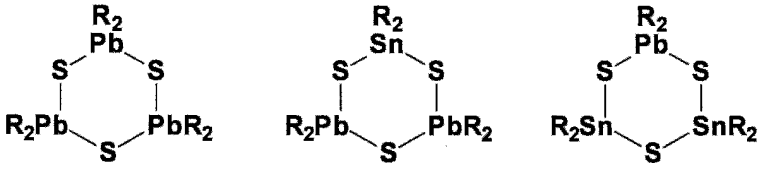


Scheme 43

9.2. Sulphur compounds

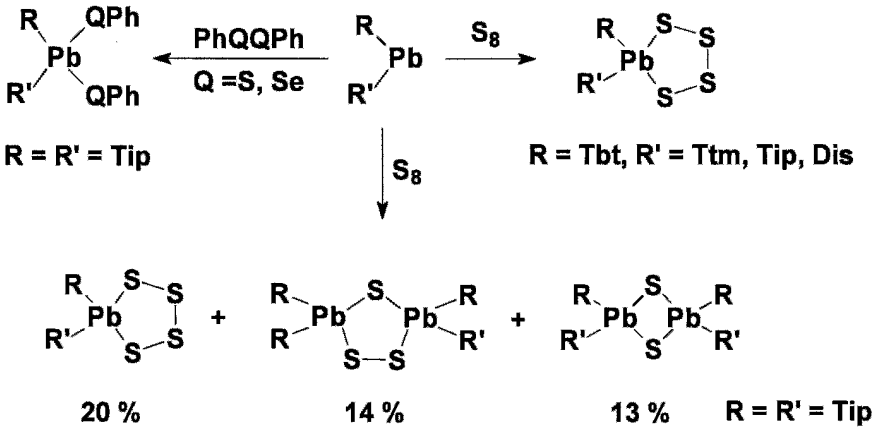
Bis(triphenyllead) sulfides and selenides are prepared from triphenyllead chloride and sodium sulfide and selenide, respectively. The compounds $R_3PbQPbR_3$ (Q = S, Se) are monomeric. With diphenyllead dichloride the same compounds are obtained following a disproportionation [131].

Diorganolead sulphides are cyclic trimers, $(R_2PbS)_3$ (R = Ph, 2- and 4-MeC_6H_4) [132, 133] containing a six-membered Pb_3S_3 ring with twist boat conformation. They undergo redistribution/equilibration reactions with diorganotin analogues $(R_2SnS)_n$ (n = 2, 3) to form the complete series of mixed lead-tin-sulphfur six-membered rings $R_6Pb_{3-x}Sn_xS_3$ (Scheme 44) [134]. Redistribution between cyclic trimers with different substituents at lead, *i.e.* $(R_2PbS)_3$ and $(R'_2PbS)_3$ with formation of the mixed substituent derivatives also occurs [133].



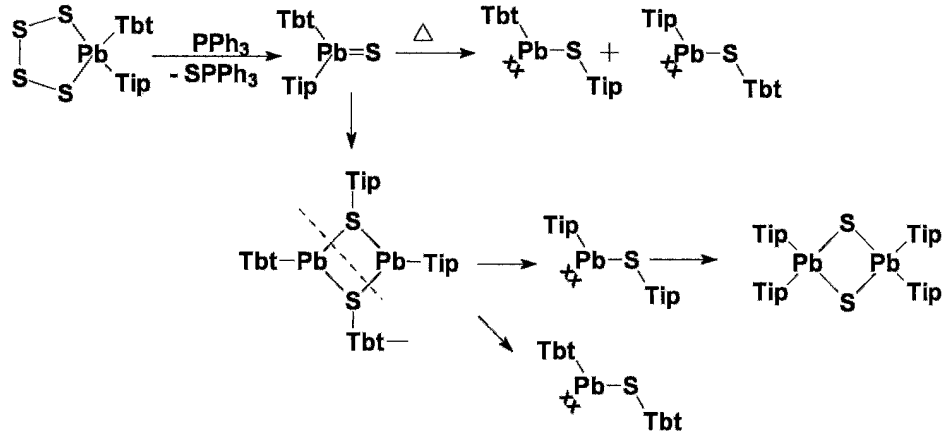
Scheme 44

The reactions of plumbylenes with elemental sulphur are a rich source of cyclic lead-sulphur compounds. Thus, $PbTip_2$ reacts with S_8 to form three different lead-sulphur rings, whereas PbTbtR forms only the five-membered PbS_4 ring derivatives (R = Ttm, Tip and Dis) but $PbTbt_2$ forms $TbtS_6Tbt$ and $TbtS_8Tbt$ [135] (Scheme 45).



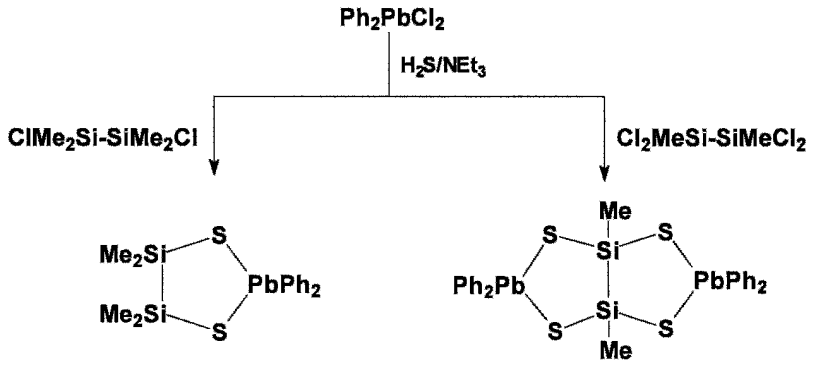
Scheme 45

A careful investigation of the desulphurization of (Tbt)(Tip)PbS_4 with triphenylphosphine revealed the formation of TbtPb-STbt (20%) and dimeric $[Tip_2PbS]_2$ (32%), among other products (TbtSH, TbtSSTbt, TbtH, TipH and $SPPh_3$). The formation of these products is explained by a mechanism involving transient monomeric (Tbt)(Tip)Pb=S plumbanethione [136] (Scheme 46).



Scheme 46

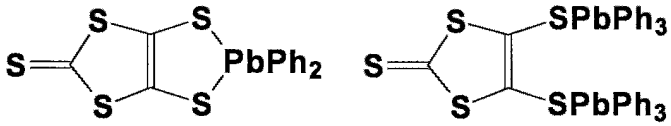
Bicyclic organolead compounds containing Pb-S or Pb-Se bonds and also silicon in fused five-membered rings have been prepared according to the reactions shown in Scheme 47. The bicyclic structure of $Ph_2PbS_2(MeSiSiMe)S_2PbPh_2$ was confirmed by X-ray diffraction. The five-membered rings display envelope conformation and Pb-S bonds are 2.4995(11) and 2.4915(11) Å [137].



Scheme 47

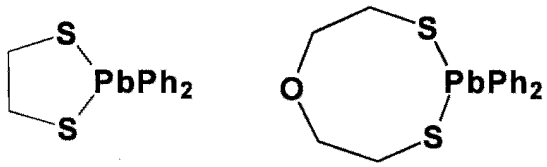
Organolead thiolates, Ph_3PbSR (R = Me, Ph) are monomeric [138], contrary to expectations, since many other organolead-sulphur compounds are self-assembled into supramolecular structures (*vide infra*).

Other interesting organolead compounds with lead-sulphur bonds include dithiocarbamates [139], monothiocarboxylates, *e.g.* $Ph_3PbS(O)CC_6H_4Me$-4 [140], dithiocarboxylates, $Ph_nPb(S_2CR)_{4-n}$ (n = 2 and 3) [141], and dithiophosphates, *e.g.* $Ph_3PbS_2P(OEt)_2$ and $Ph_2Pb[S_2P(OCH_2Ph)_2]_2$ [142], dithiophosphinates, *e.g.* $Ph_2Pb(S_2PPh_2)_2$ [143], and $Me_3Pb(S_2PR_2)$ (R = Me, Et, Ph) [144], dithioarsinates [145], 1,3-dithiole-2-thione-4,5-dithiolates $C_3S_3(S_2PbPh_2)$ [146], $C_3S_3(SPbPh_3)_2$ (Scheme 48) [147], and toluene-3,4-dithiolates and 1,2-dimercaptobenzene derivatives [148]. Some are monomeric, molecular compounds, others are self-assembled into supramolecular structures via secondary Pb···S interactions.



Scheme 48

Heterocycles containing S-PbR_2-S fragments are also known, *e.g.* five-membered $Ph_2Pb(SCH_2)_2$ [149], and eight-membered $Ph_2Pb(SCH_2CH_2)_2O$ [150] (Scheme 49).



Scheme 49

9.3. Selenium and tellurium compounds

Relatively few organolead-selenium compounds have been investigated. Among the newest ones, the $Me_3PbSeC_6F_5$ derivative is mentioned. It was prepared from Me_3PbCl with C_6F_5SeLi [151]. Selenocarboxylates, $R_nPb(SeOCR)_{4-n}$ [152], and diselenophosphates, $Ph_3PbSe_2P(OR)_2$ [153], have been also reported. A series of selenium- and tellurium-lead compounds $Ph_3PbQOCR$ (Q = Se, Te; R 1-adamantanyl, 4-MeC_6H_4, 4-ClC_6H_4) have been prepared from Ph_3PbCl and sodium carbotelluroates [154].

10. ORGANOLEAD COMPOUNDS CONTAINING Pb-N, Pb-P, Pb-As and Pb-Sb BONDS

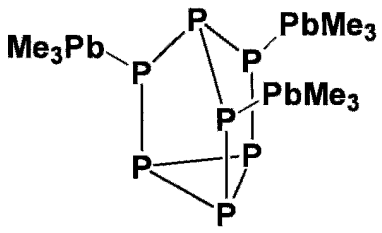
10.1. Nitrogen compounds

Organolead compounds with lead-nitrogen bonds have been little investigated due to their instability. The ammonolysis of triorganolead halides in liquid ammonia with KNH_2 stops at one of the successive stages R_3PbNH_2, $R_3PbNHPbR_3$ or $N(PbR_3)_3$, depending on the bulkiness of the organic group [155]. Amino derivatives, *e.g.* $R_nPb(NR'_2)_{4-n}$, can be prepared from halides by metathesis with lithium or sodium amino derivatives [156]. Very bulky ligands stabilize amino derivatives of organolead(II). Thus, $[ArPbNH_2]_2$ with Ar = C_6H_3-2,6-(C_6H_2-2,4,6-Pr^i_3)$_2$ is formed in the reaction of ArPbBr with $LiNH_2$ in diethyl ether. With liquid ammonia only the adduct $ArPbBr(NH_3)$ is obtained, no substitution taking place. These are the first organolead(II) amine complexes [157].

The sulfonamide derivatives, $R_3Pb\text{-}N(SO_2X)_2$ (R = Me, Ph; X = Me, F), prepared from organolead chlorides with $AgN(SO_2X)_2$, are associated in the solid state into a chain-like supramolecular structure through Pb···O intermolecular bonds [158].

10.2. Phosphorus, arsenic and antimony compounds

Some compounds containing lead-phosphorus bonds, $(Ph_3Pb)_nPPh_{4-n}$, can be obtained by condensation of Ph_3PbCl with phosphines Ph_nPH_{3-n}, in the presence of triethylamine, and by chlorotrimethylsilane elimination between Ph_3PbCl and $Bu^tPhPSiMe_3$ or by metathesis between Ph_3PbCl with $LiP(SnPh_3)_2$ [159]. An uncommon lead-phosphorus compound is the P_7 cage derivative $P_7(PbMe_3)_3$ [160] (Scheme 50).

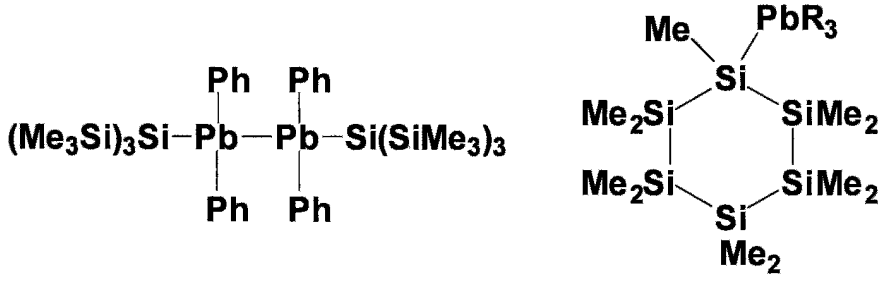


Scheme 50

Exotic arsino- and stibino derivatives, $E(PbR_3)_3$ and Ph_3PbEPh_2 (E = Sb, Bi) have been obtained from Ph_3PbCl with EH_3 hydrides in the presence of triethylamine or with $NaEPh_2$ in liquid ammonia [161].

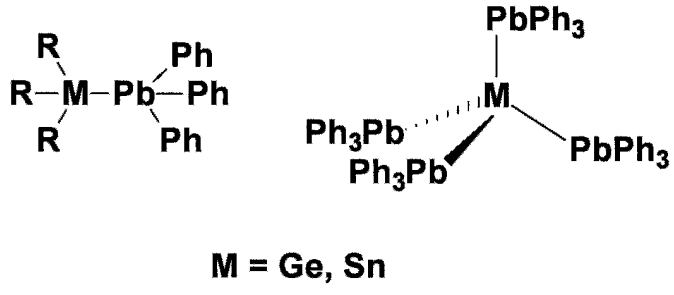
11. ORGANOLEAD COMPOUNDS CONTAINING Pb-Si, Pb-Ge, Pb-Sn BONDS

Organolead moieties can be connected to other Group 14 elements. A linear compound with Pb-Si bonds, $(Me_3Si)_3Si\text{-}PbPh_2PbPh_2\text{-}Si(SiMe_3)_3$ [162], and triorganoplumbyl derivatives of cyclohexasilane, $R_3PbSi_6Me_{11}$ (R = Me, Ph) have been reported [163] (Scheme 51).



Scheme 51

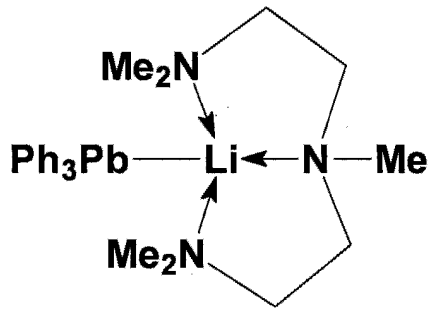
Hexaphenyldilead is cleaved by lithium metal in THF to form a lead-lithium bonded compound, Ph_3PbLi [164], which is a useful reagent for the synthesis of organolead-germanium and -tin compounds, $Ph_3Pb\text{-}MR_3$ (M = Ge, Sn) [156, 165], and $M(PbPh_3)_4$ (M = Ge, Sn) in metathesis reactions with metal chlorides [12]. The molecular structures of $R_3Ge\text{-}Pb(C_6H_4Me\text{-}4)_3$ (R = Ph, C_6H_4Me-4) and $Ph_3Sn\text{-}PbPh_3$ have been determined by X-ray diffraction [165] (Scheme 52).



Scheme 52

12. ORGANOLEAD COMPOUNDS CONTAINING Pb-M BONDS

Triorganoplumbyl derivatives of alkali metals can be prepared and are useful starting materials for the synthesis of numerous other organolead compounds. Some examples have already been cited above. Triphenylplumbyllithium, $LiPbPh_3$, is obtained by cleavage of $Ph_3PbPbPh_3$ with lithium metal [166]. The plumbyllithium compounds can be stabilized by coordination *e.g.* with pentamethyldiethylenetriamine [167] (Scheme 53).

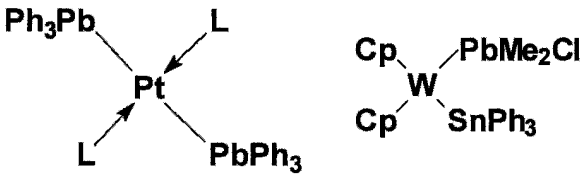


Scheme 53

Trialkyl- and -arylplumbylmagnesiumhalides, $R_3PbMgCl$, are formed in the reaction of Grignard reagents with $PbCl_2$ in THF, which proceed further to hexaaryldilead or tetraalkyllead compounds [168]. The preparation of tris[(trimethylsilyl)methyl]plumbylmagnesium chloride, $(Me_3SiCH_2)_3PbMgCl$ and its use as reagent in the synthesis of several organolead compounds has been described [169].

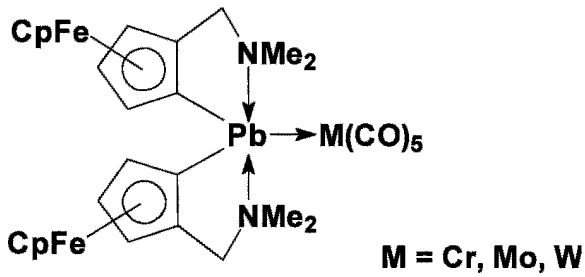
Triorganoplumbyl groups may act as ligands and numerous transition metal complexes containing lead-metal bonds have been described (see reference [170] for a recent review).

Most frequently for their synthesis is used the metathesis reaction of organolead halides with metal carbonyl, cyclopentadienylmetalcarbonyl and other organometallic anions. Among the compounds of this class, $Cp_2W(SnPh_3)(PbMe_2Cl)$ [171], $Ph_3PbV(CO)_5$ [172], $Me_3PbMn(CO)_5$ [173], $Ph_3PbRe(CO)_5$ [174], $Me_3PbCo(CO)_4$, $CpM(CO)_3PbMe_3$ (M = Cr, Mo, W), [175 - 178], $CpFe(CO)_2PbMe_3$, [179, 180], $[M(Ph_3Pb)_2(PPh_3)_2]$ (M = Pd, Pt) [181] are mentioned. The platinum complex *cis*-$[(PPh_3)_2Pt(PbPh_3)Ph]$ was prepared from $[(PPh_3)_3Pt(\eta^2\text{-}H_2C{=}CH_2)]$ with a benzo-1,3.2-diazaborolidine triphenyllead derivative (Scheme 54) [182].



Scheme 54

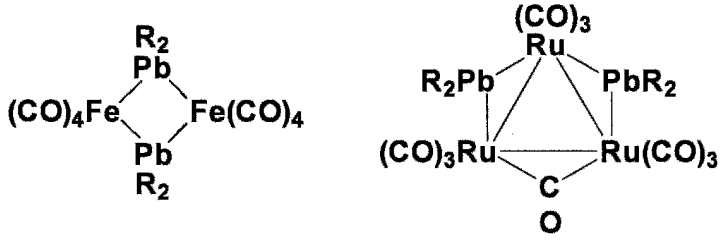
Plumbylene donor metal complexes, *e.g.* chromium, molybdenum and tungsten derivatives of an internally stabilized bis(dimethylaminomethyl-ferrocenyl)plumbylene have been prepared and structurally characterized [183] (Scheme 55).



Scheme 55

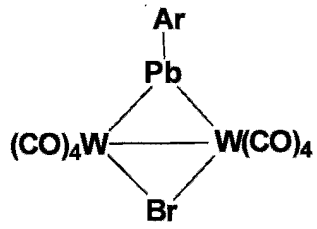
Compounds containing a M-Pb-M trimetallic sequence are known, *e.g.* $(CO)_5Mn\text{-}PbPh_2\text{-}Mn(CO)_5$ [184] and $Cp(CO)_3Mn\text{-}PbEt_2\text{-}Mn(CO)_3Cp$ [185].

Four-membered ring compounds containing lead and iron, $[Et_2PbFe(CO)_4]_2$ [180, 186] and clusters incorporating organolead moieties, *e.g.* $Ru_3(CO)_9(\mu\text{-}CO)_2(PbR_2)$ and $Ru_3(CO)_9(\mu\text{-}CO)(PbR_2)_2$ [187] are only two examples (Scheme 56) illustrating the great diversity of organolead-transition metal compounds.



Scheme 56

An uncommon bridging mode of a plumbylyne moiety is observed in the tungsten complex $[W(CO)_4]_2(\mu_2\text{-}Br)(\mu_2\text{-}PbAr)$ obtained from $W(CO)_5(THF)$ with $[ArPbBr]_2$ (Ar = $C_6H_3Trip_2$-2,6) [108] (Scheme 57).



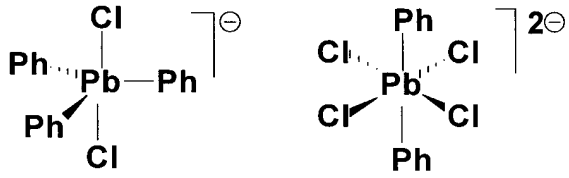
Scheme 57

13. HYPERVALENT ORGANOLEAD COMPOUNDS

A series of organolead complexes, containing more than four lead-element bonds (of which some are Pb-C bonds) are described as "hypervalent". These are formed by coordination of additional neutral or anionic ligands to a normal organolead derivative. Additional halide anions frequently coordinate to organolead halide molecules to increase the coordination number of lead change the coordination geometry with formation of "hypervalent" anionic species.

A five-coordinate anion, $[Ph_3PbCl_2]^-$ is formed in the reaction of $Ph_2Pb(C_3S_5)$ with $[NMe_4]Cl$ and the trigonal bipyramidal coordination geometry [axial Pb-Cl 2.7312(14) Å, axial Cl-Pb-Cl 176(2)°, equatorial Cl-Pb-C 87.8(2)-92.1(10)°] was established by single crystal X-ray diffraction [188].

In the reactions of Ph_2PbCl_2 with $[PR_4]Cl$ (R = Bu^n and Ph) two different complex anions (Scheme 58) were formed: a trigonal bipyramidal, five-coordinate complex in the salt $[PPh_4][Ph_3PbCl_2]$ and an octahedral six-coordinate complex in the salt $[PBu^n{}_4]_2[Ph_2PbCl_4]$. In the trigonal bipyramidal anion $[Ph_3PbCl_2]^-$ the phenyl groups occupy equatorial positions [cis-equatorial C-Pb-C 113.71(17)°, 121.84(15)° and 124.39(15)°; trans-axial Cl-Pb-Cl 177.70(4)°. Pb-Cl . 2.727(1) Å] and in the distorted octahedral dianion $[Ph_2PbCl_4]^{2-}$ the phenyl groups are in trans-axial positions [*trans* Cl-Pb-Cl 178.00(3)° and 179.00(3)°, *trans* C-Pb-C 177.35(13)°; Pb-Cl 2.6968(9)-2.7392(9) Å] [189].

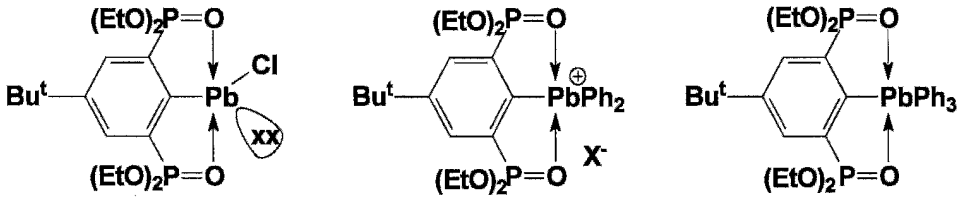


Scheme 58

Organolead(IV)-fluoride complexes have been investigated in solution and their stability constants have been determined [190].

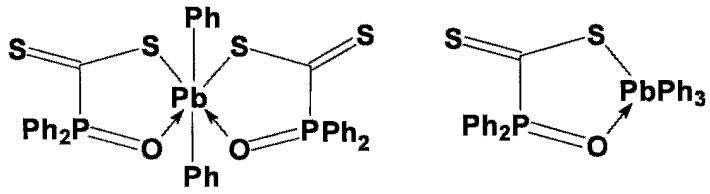
Coordination of neutral molecules is illustrated by the formation of Lewis base complexes of alkyllead(IV) chlorides, R_3PbCl (R = Me, Et) with tetramethylene sulfoxide, dimethylformamide and dimethylacetamide [191] reported as early as 1964. The structures of triphenyllead(IV) bromide adduct with triphenylphosphine oxide, $Ph_3PbBr{\cdot}OPPh_3$ [192], and of triphenylarsine oxide adduct of triphenyllead chloride, $Ph_3PbCl{\cdot}OAsPh_3$ have been confirmed by X-ray diffraction; both are five-coordinate, trigonal bipyramidal complexes with Pb-X and Pb-O bonds in axial positions and the Pb-C bonds in equatorial positions [193]. Similarly, in the five-coordinate complex $Ph_3PbBr{\cdot}L$ (L = cyclopropenone) the halogen and oxygen are in axial positions [194]. Other structurally characterized complexes are $Ph_3PbCl{\cdot}HMPT$ (five-coordinate, trigonal bipyramidal) and $Ph_2PbCl_2{\cdot}2L$ with L = HMPT, DMSO (octahedral) [195]. Obviously, this class of compounds can be much extended.

Lead(II) organometallic halide and thiophenyl derivatives, 4-Bu^t-2,6-$C_6H_2[P(O)(OEt)_2]_2PbX$ (X = Cl, SPh) with intramolecular chelation from *ortho*-phosphoryl groups and pseudo-trigonal bipyramidal coordination at lead (displaying a stereochemically active lone pair) (Scheme 59) was prepared by traditional organolithium synthesis and structurally characterized. In spite of the apparent crowding around lead, the compounds are assembled into supramolecular chain-like arrays through Pb-X···Pb bonds. Cationic [3+2] coordinate, and neutral [4+2] coordinate organolead(IV) derivatives with the same substituent have also been obtained and structurally characterized [196].



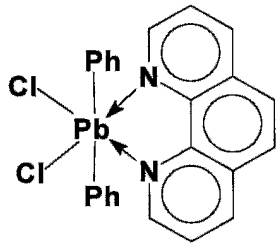
Scheme 59

In organolead phosphinoyl dithioformates the ligand is bidentate chelating through sulphur and oxygen and as a result in $Ph_2Pb[S_2CP(O)Ph_2]_2$ the lead atom is six-coordinate, with a distorted octahedral coordination geometry and in $Ph_3Pb[S_2CP(O)Ph_2]$ the metal is five-coordinate, trigonal bipyramidal [197] (Scheme 60).



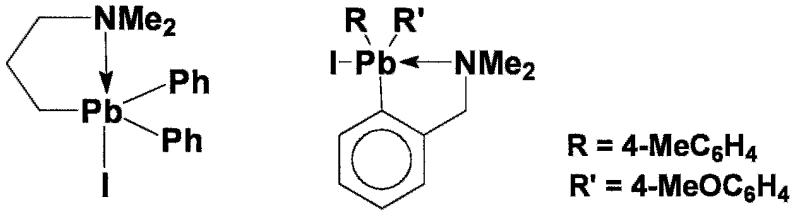
Scheme 60

Six-coordination (octahedral geometry) is conveniently achieved by using ortho-phenanthroline as bidentate chelating ligand in diorganolead halide complexes [198] (Scheme 61).



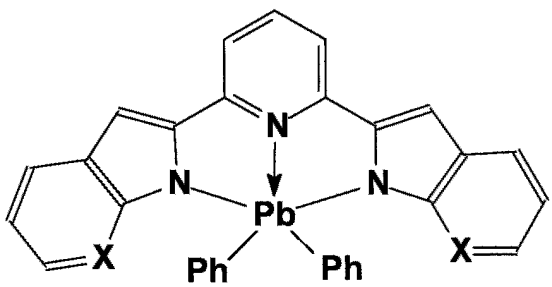
Scheme 61

Intramolecular N→Pb coordination leading to cyclization of N-dimethylamino(propyl) derivatives, $Me_2N(CH_2)_3PbPh_2X$ (X = Cl, Br, I) increases the coordination number of lead and generates a trigonal bipyramidal geometry as confirmed by an X-ray diffraction analysis of the iodo derivatives and ^{207}Pb NMR for all three halogeno derivatives [199]. In aromatic derivatives *ortho*-(dimethylamino)methyl substituents have a similar effect, with the formation of the same five-membered chelate ring [200] (Scheme 62).



Scheme 62

Deprotonated bis(indolylpyridine) (bip) and 2,6-bis[2'-(7-azaindolyl)] pyridine (bap) coordinate as tridentate chelating ligands to Ph_2Pb^{2+}, to form five-coordinate distorted trigonal bipyramidal complexes Ph_2Pb(bip) and Ph_2Pb(bap) with three Pb-N bonds and two phenyl groups [201] (Scheme 63).



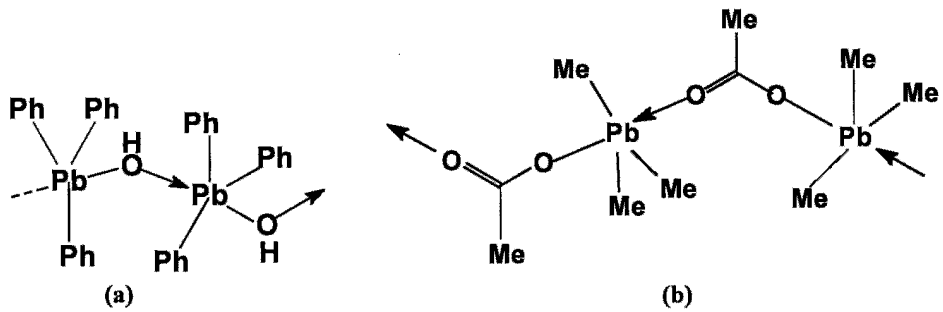
Scheme 63

14. SUPRAMOLECULAR-SELF ASSEMBLY OF ORGANOLEAD COMPOUNDS

In many cases organolead molecules self-assemble into "*well-defined, discrete oligomolecular species that result from the intermolecular association of a few components*" called *supermolecules*, or into supramolecular assemblies also called *supramolecular arrays*, which are "*polymolecular entities that result from the spontaneous association of a large undefined number of components*" [202]. This process frequently takes place in Main group organometallic chemistry [203].

Some examples were already cited above, where association of some species was mentioned. A systematization according to the bonding types (dative coordinate or Lewis acid-base interactions, secondary bonding, hydrogen bonds, ionic interactions, π-bond interactions) leading to supramolecular self-assembly is attempted below.

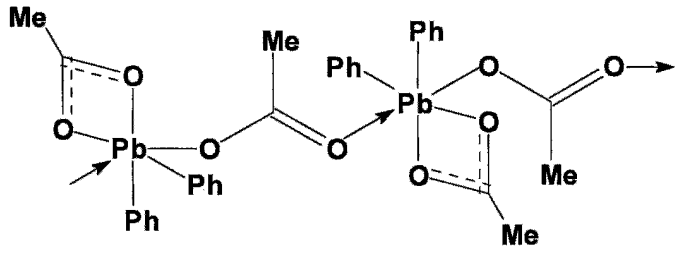
Self-assembly through *dative coordinate bonds* occurs in organolead hydroxides, alkoxides, carboxylates and other oxygen containing anionic ligands. Differing lead-oxygen interatomic distances are observed, with shorter intramolecular bonds (polar covalent Pb-O) and slightly longer intermolecular distances (dative coordinate O→Pb). Thus, triphenyllead(IV) hydroxide, Ph_3PbOH, forms chain-like supramolecular arrays [204] (Scheme 64a). Trimethyllead acetate, $Me_3Pb(OOCMe)$ forms infinite zig-zag chains in which trigonal planar $PbMe_3$ groups are bridged by acetate groups, leading to pentagonal bipyramidal coordination of lead (Scheme 64b), with Pb-O 2.327 Å and O→Pb 2.555 Å, axial O-Pb-O 169.7° [205]. Similar self-assembly occurs in trimethyllead furoate, $Me_3Pb(OOCC_4H_3)$ (with Pb-O 2.353 Å and O→Pb 2.534 Å; O-Pb-O 169.4°) [206].



Scheme 64

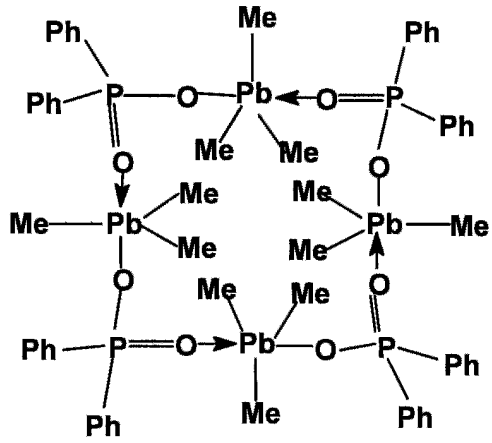
Diphenyllead(IV) diacetate, $Ph_2Pb(OOCMe)_2$, forms a chain-like supramolecular array with one acetate group chelating and one bridging

(Scheme 65), displaying different interatomic distances: Pb-O 2.348 Å and O→Pb 2.547 Å in the chain and 2.354 Å and 3.364 Å in the chelate ring [207].



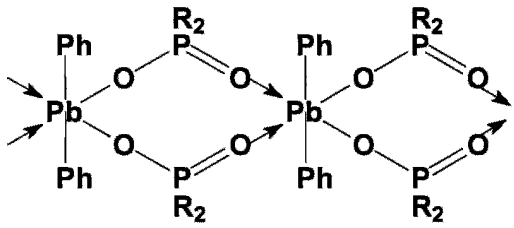
Scheme 65

A tetrameric supermolecule is formed by self-assembly of trimethylead(IV) diphenylphosphinate, which forms a sixteen membered macrocyclic system $[Me_3Pb(O_2PPh_2)]_4$ with bridging diphosphinato groups (Scheme 66). The lead atoms are in trigonal bipyramidal coordination with equatorial Pb-Me groups and axial Pb-O bond in the range 2.373(6)-2.402(6) Å, axial O-Pb-O angles 174.7(2)-177.6(3)° [208].



Scheme 66

The diphenyllead(IV) phosphinates $Ph_2Pb(O_2PR_2)_2$ (R = Me, Ph) form chain-like supramolecular arrays, with doubly bridging O-PR_2-O ligands and eight-membered $Pb_2O_4P_2$ rings connected in a spiro fashion [209] (Scheme 67).

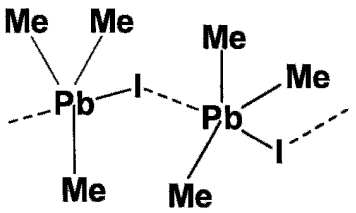


Scheme 67

Organolead(IV) arsinates, *e.g.* $Ph_3PbO_2AsMe_2$, and arsonates, *e.g.* $R_3PbO_2As(O)Me$, have also been reported [210], but no crystal structure analysis on such compounds has been performed so far. Very likely, there is a structural similarity with phosphinates and phosphonates.

Self-assembly through *secondary bonds (or soft-soft interactions*) occurs in organolead halides. Some examples of organolead(II) halides were cited above. This type is observed in lead compounds containing heavier halogens or chalcogens. Since the intermolecular interatomic distances cover rather broad limits sometimes it is difficult to draw a line between dative coordinate and secondary bonds, especially in the case of lead-oxygen bonds; therefore the distinction between the two types may seem arbitrary.

Triorganolead(IV) halides with organic groups of small or moderate size are always self-assembled into supramolecular arrays. This was first suggested on the basis of vibrational spectra of tri- and diorganolead(IV) halides [211] and then confirmed by X-ray diffraction. Typically, trimethyllead iodide molecules, Me_3PbI, are self-assembled into zig-zag chains (Scheme 68) with linear I-Pb···I and bent Pb-I···Pb fragments of trigonal bipyramids with the methyl groups in equatorial positions (Pb-I 3.038 Å, Pb···I 3.360 Å; I-Pb···I 176.25°) [212].

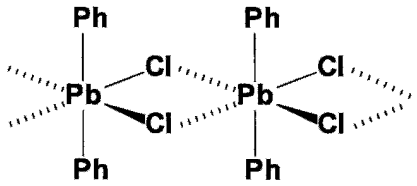


Scheme 68

Trimethyllead(IV) chloride, Me_3PbCl, also forms zig-zag chains of distorted trigonal bipyramids (Pb-Cl 2.764 Å and Pb···Cl 2.814 Å) [213]. Similar chain structures of trigonal bipyramids have triphenyllead chloride Ph_3PbCl (axial Pb-Cl 2.706 Å, Pb···Cl 2.947 Å, Cl-Pb···Cl 179.5°), triphenyllead(IV)

bromide Ph_3PbBr (axial Pb-Br 2.852 Å, Pb···Br 3.106 Å, Br-Pb···Br 173.8°) [214], and benzyldiphenyllead(IV) bromide (axial Pb-Br 2.885 Å, Pb···Br 2.985 Å, Br-Pb···Br 173.61°) [215]. Organolead halides with large organic groups, like tri(mesityl)lead chloride, are monomeric, un-associated molecular compounds in solid state [216].

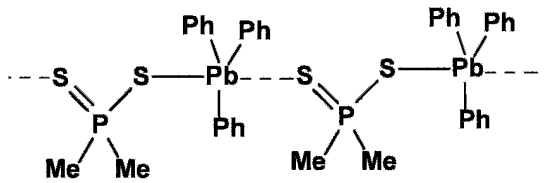
Diphenyllead(IV) chloride, Ph_2PbCl_2, is self-assembled into chain-like arrays with double halogen bridges, six-coordinate octahedral lead and axial phenyl groups [217] (Scheme 69).



Scheme 69

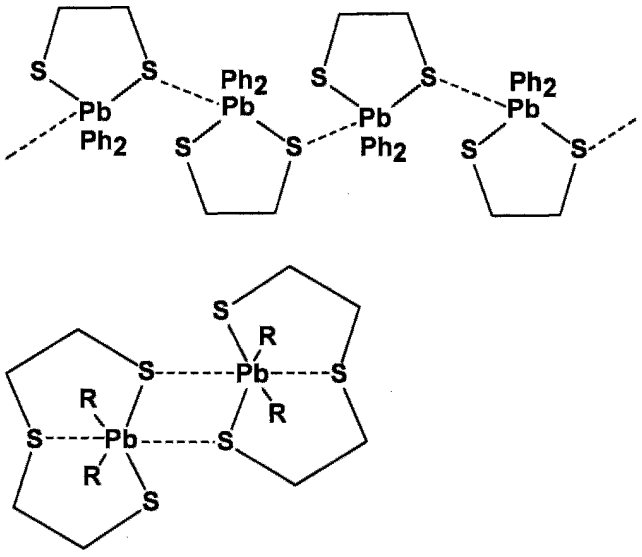
The self-assembly of 1-nitro-2-triphenylplumbylthiolato-benzene, $C_6H_4(1\text{-}NO_2)(2\text{-}SPbPh_3)$ through weak intermolecular lead-oxygen interactions reflected in long Pb···O interatomic distances [3.180(5) Å] can be described as being due to secondary interactions; the lead atom is five-coordinate with oxygen (of a nitro group) and sulphur in axial positions [S-Pb···O angle 173.96(9)°] and a supramolecular chain-like array results [218].

Some sulphur-containing organolead compounds form supramolecular structures by self-assembly through Pb···S secondary bonds. Triphenyllead(IV) dimethyldithiophosphinate, $Ph_3PbS_2PMe_2$, is one of such compounds. It forms chain-like arrays (Scheme 70) with bridging dithiophosphinato ligands (Pb-S 2.708 Å, Pb···S 3.028 Å) [219] and diphenyllead(IV) bis(dibenzyldithiophosphate), $Ph_2Pb\{S_2P(OCH_2Ph)_2\}_2$, was shown to dimerize through weaker Pb···S secondary bonds (3.69 Å) [142].



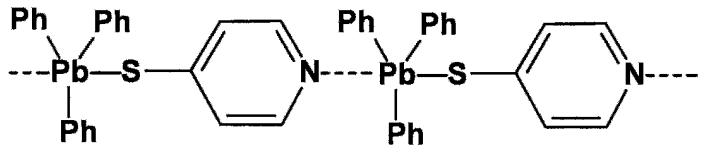
Scheme 70

The cyclic diphenyl dithiaplumbolanes, $Ph_2Pb(SCH_2)_2$, are self-assembled into linear supramolecular arrays through weak secondary bonds (3.55 Å) which are still shorter than Van der Waals distances [149], and 5,5-diphenyl-1,4,6,5-trithiaplumbocane is associated into dimeric supermolecules with intermolecular Pb···S distances of 3.750 Å, compared with intra-ring distances of 2.514 and 2.519 Å [150] (Scheme 71).



Scheme 71

Some supramolecular self-assembly may occur through Pb···N secondary bonds. Thus, chain-like arrays were found in azides, Me_3PbN_3 and Ph_3PbN_3 [220], cyanide Me_3PbCN [221], and triphenyllead(IV) pyridine-4-thiolate, $Ph_3PbSC_5H_4N$ [222] (Scheme 72).

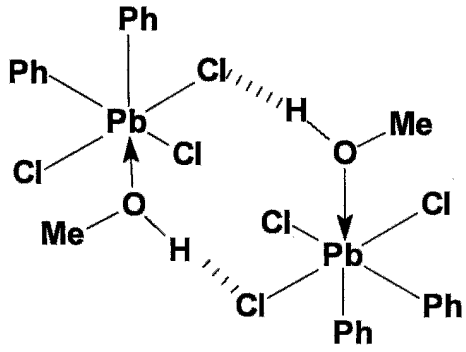


Scheme 72

Organolead thiocyanates, *e.g.* R_3PbNCS (R = Me, Ph) and $Ph_2Pb(NCS)_2$ were shown by infrared spectroscopy to be associated through NCS bridges [223] but no crystal structure analysis is available so far.

Trimethyllead hexacyanometallates, $[M(CN)_6(PbMe_3)_4]$ (M = Fe, Ru, Co) form tridimensional supramolecular architectures with extended channels which afford formation of host-guest structures [224].

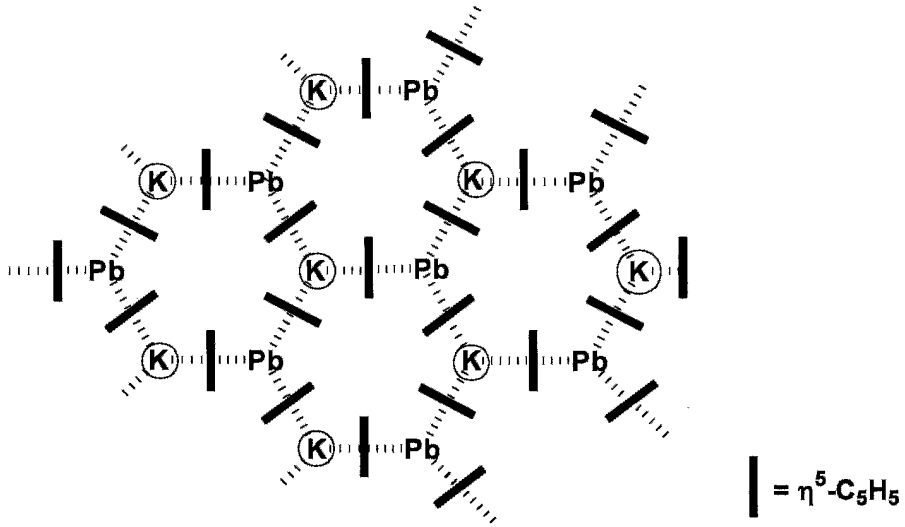
Self-assembly through *hydrogen bonds* has been rarely reported in organolead compounds. A nice example is the dimeric supermolecule (Scheme 73) formed by hydrogen bonds of coordinated methanol in the $[Ph_2PbCl_3(MeOH)]^-$ (associated with a cationic supramolecular dimer formed through secondary Pb···Cl bonds, $[Ph_2PbCl(HPyTSC)]_2$, containing a coordinated thiosemicarbazone) [225].



Scheme 73

The bis(imidazole) complex $Ph_2PbCl_2(C_3H_4N_2)_2$ forms two isomers, with phenyl groups in *cis*- and *trans*-positions; both are self-assembled in the solid state through N-H···Cl hydrogen bonds, the *cis* isomer forming supramolecular chains and the *trans* isomer forming two-dimensional layers [226].

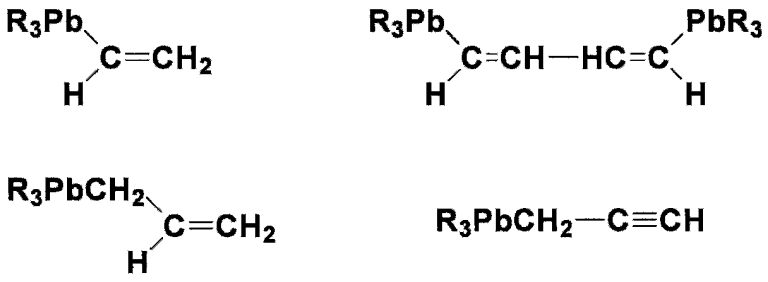
Supramolecular self-assembly through *π-bonding interactions* was mentioned above in the presentation of plumbocenes $Pb(\eta^5\text{-}C_5H_5)_2$ [62, 63, 64]. Another example is that of $K^+[PbCp_3]^-$ in which the honeycomb bidimensional supramolecular architecture [80] is formed through charge-assisted π-bonding interactions, *via* alternation of potassium cations with the organolead anions (Scheme 74).



Scheme 74

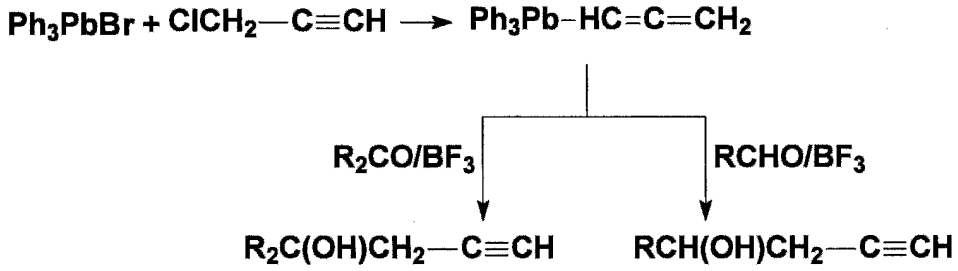
15. SELECTED USES

The major use of organolead compounds was as antiknock gasoline additive, the major player being tetraethyllead. The whole dramatic story of rise and fall of this compound, which is gradually eliminated because of environment pollution concerns [227], is beautifully told in a two-part comprehensive review article [2] (see also Chapter 1, 1.1 and 1.3). Other organolead compounds studied as candidates for additives to raise the octane rating of fuels were some organolead alkenes and alkynes [228, 229] (Scheme 75).



Scheme 75

Few other major uses of organolead compounds worth mentioning. Among these is the use of organolead compounds as reagents in organic synthesis. Thus, organolead reagents can be used for transformation of propargyl to allenyl derivatives [230] and this chemistry has been used as an allenyl transfer reagent to aldehydes and ketones [231] (Scheme 76).



Scheme 76

The use of organolead(IV) triacetates in organic synthesis is also worth mentioning. These compounds are versatile reagents for the electrophilic arylation, vinylation and alkynylation of various carbon nucleophiles, and this served for the synthesis of a series of natural products and medical drugs [232, 233].

16. STRUCTURAL ^{207}Pb NMR SPECTROSCOPY.

The molecular structure determination of organolead compounds is best served by single crystal X-ray diffraction. The solid state structures determined by this technique have been cited above for most classes of organolead compounds. One of the major techniques used specifically for the characterization of organolead compounds is ^{207}Pb NMR spectroscopy, which is now routinely used for the solids as well as in solution state. The ^{207}Pb isotope is NMR active because it has a nuclear spin of ½, and natural abundance of 22.6 %. Because of its high abundance, it has sensitivity of about 12 times higher than the ^{13}C NMR and 0.9 % of that of the proton. The variations in chemical shift values for divalent and tetravalent organolead compounds are large and a very broad chemical shift range from +11000 to -7000 ppm has been observed. A number of reviews dealing with ^{207}Pb NMR were appeared over the years [3e, 234]. The trends observed in ^{207}Pb NMR broadly parallels with the trends observed in ^{119}Sn NMR spectra of the similar organotin compounds.

16.1. Lead (II) compounds

The ^{207}Pb NMR chemical shifts for divalent lead compounds exhibit strong downfield shifts covering a wide range from 11000 to 3000 ppm consistent with the low coordination number of the lead. The selected examples of divalent lead compounds are provided in Table 1. There seems to be no correlation between the chemical shift values and structural parameters like bond angles, bond lengths of the divalent lead compounds. An increase in coordination number of lead from two to three which occurs upon adduct formation with donor ligands result in large >1000 ppm upfield chemical shifts. The effect of the incremental increase in coordination number is quite visible in a series of low-valent compounds [53], for example, one-coordinated lead cation Ar^*Pb^+ (Ar^* = 2,6 $Trip_2$-C_6H_3) with a resonance at 8974 ppm, is shifted 1554 ppm to the low field from its 2-coordinated precursor, Ar*PbMe (chemical shift at 7420 ppm) and a further increase in coordination number to three in the pyridine adduct of the cation $Ar^*Pb^+(py)_2$ (^{207}Pb NMR 4764 ppm) results in upfield shift of 4210 ppm from the one-coordinated cation. This trend is also observed in plumbylene-carbene adducts. The chemical shifts of the diplumbenes, compounds with formal Pb=Pb double bonds; in solution are in close proximity with the plumbylenes indicating they completely dissociate into monomeric plumbylenes at room temperature exhibiting the weak bonding between the lead atoms.

16.2. Lead (IV) compounds

The chemical shift values of lead(IV) compounds of the type R_nPbX_{4-n} are generally more sensitive to the chemical environment, particularly the coupling constants, ^nJ(Pb-C), the wide lead shift range allows a clear distinction between the closely related species. The chemical shifts are dependent upon concentration, the solvent and the nature of inorganic groups X. A high field shift occurs in donor solvents where increases in coordination number can be expected. As the electron withdrawing power of the X increases, the lead atom becomes more deshielded and the chemical shifts move to low field. The replacement of methyl groups in Me_4Pb by unsaturated organic groups such as vinyl, alkynyl or aryl groups leads to high field shift of ^{207}Pb resonance. Few selected examples of tetravalent lead compounds are provided in Table 2.

A series of organolead derivatives of the transition metals have been investigated using ^{207}Pb NMR [175, 242]. The presence of a transition metal unit (ML) induces a low field shift when compared to the corresponding methyl derivative; *i.e.* R_3ML *vs.* R_3PbMe for ML = $CpFe(CO)_2$, $CpMo(CO)_3$ and $Mn(CO)_5$ and $Co(CO)_4$ and some representative examples are presented in Table 2. Within a group of the transition metals, *e.g.* Cr, Mo, W, there is a progressive shift to high field as one descends the group such that the third row

transition metal tungsten produces a high field shift when compared to the organolead analog. In a similar manner, increasing amounts of the metal substituent cause a further upfield shift for W but low field shift for Mo. The effect of solvent is small in this group of organoleads for the solvent series C_6D_6, CH_3OH and C_5H_5N.

The various trends noted above parallel the related data with organotin derivatives using ^{119}Sn NMR with the extra feature that the range of chemical shifts are about three times more sensitive to the metal substituent for the lead nucleus.

Table 1.
^{207}Pb NMR data for selected divalent organolead compounds

Compound	Chemical shifts (ppm)	Reference
$(Me_3Si)_3SiPbC_6H_3$-2,6-$Trip_2$	10745	[112]
$(Me_3Si)_3SiPbC_6H_3$-2,6-Mes_2	10510	[46d]
$Pb[C(SiMe_3)_2SiMe_2CH_2]_2$	10050	[52]
$Pb(Tbt)_2$	9751	[46a]
$Pb[CH(SiMe_3)_2]_2$	9112	[45,46d]
$Pb[C_6H_2$-2,4,6-$\{CH(SiMe_3)_2\}_3]_2$	8971	[46a]
TbtPbTip	8888	[46a]
$TbtPbCH(SiMe_3)_2$	8884	[46a]
$TbtPbC_6H_2$-2,4,6-$(CH_2SiMe_3)_3$	8873	[46a]
$Pb(C_6H_3$-2,6-$Mes)_2$	8844	[235]
$Bu^tPbC_6H_3$-2,6-$Trip_2$	7853	[235]
$MePbC_6H_3$-2,6-$Trip_2$	7420	[108]
$Pb(C_6H_2$-2,3,5-Me_3-6-$Bu^t)_2$	6927	[55]
$PhPbC_6H_3$-2,6-$Trip_2$	6657	[108]
$Mes^*PbCH_2CMe_2C_6H_3Bu^t_2$	5067	[55]
$Pb[C_6H_2$-2,4,6 $(CF_3)_3]_2$	4878	[236]
$Pb(C_6H_2$-2, 4, 6-$Trip_3NH_2)_2$	3209	[157]

$Mes^* = C_6H_2$-2,4,6-$^tBu_3$; Tbt = C_6H_2-2,4,6-$\{CH(SiMe_3)_2\}_3$;
Tip = C_6H_2-2,4,6-$(CHMe_2)$; Trip = -C_6H_2-2,4,6-$^iPr_3$

Table 2.
^{207}Pb NMR data for selected tetravalent organolead compounds

Compound	Chemical shifts (ppm) (solvent)	Reference
Me_3PbCl	375 ($CDCl_3$)	[237]
Me_3PbCl	216 (Pyridine)	[237]
Et_3PbCl	472 ($CHCl_3$)	[238]
Ph_3PbCl	33 ($CDCl_3$)	[238]
Ph_3PbBr	-3 ($CHCl_3$)	[238]
Ph_3PbI	-131 ($CHCl_3$)	[238]
Ph_4Pb	-179 ($CDCl_3$)	[239]
Me_3Pb-$PbMe_3$	-281 (Et_2O)	[239]
Ph_3Pb-$PbPh_3$	-80 ($CHCl_3$)	[240]
Ph_3PbO_2CMe	-93 ($CDCl_3$)	[239]
Ph_3Pb-$GePh_3$	-271 (C_6D_6)	[240]
Ph_3Pb-$SnPh_3$	-256 (C_6D_6)	[240]
Me_3PbSMe	214 (Neat liquid)	[238]
$Mes_3PbCOMe$	-432 ($CDCl_3$)	[30]
Me_3Pb-PPh_2	40 (C_6H_6)	[241]
Me_3PbOMe	331 (CH_2Cl_2)	[237]
Me_3PbO_2CMe	305 (CH_3OH)	[237]
Me_3Pb-$CH{=}CH_2$	-65 (Neat liquid)	[237]
Me_3Pb-$C{\equiv}CMe$	-140 (C_6H_6)	[237]
$Co(CO)_4PbEt_3$	478 (C_6D_6)	[175]
$Mn(CO)_5PbEt_3$	231 (C_6D_6)	[175]
$CpFe(CO)_2PbMe_3$	246 (C_6D_6)	[175]
$CpCr(CO)_3PbMe_3$	360 (C_6D_6)	[175]
$CpMo(CO)_3PbMe_3$	221 (C_6D_6)	[175]
$CpW(CO)_3PbMe_3$	-100 (C_6D_6)	[175]
$CpMo(CO)_3PbPh_3$	125 (CH_2Cl_2)	[242]
$[CpMo(CO)_3]_2PbPh_2$	421 (CH_2Cl_2)	[242]
$CpW(CO)_3PbEt_3$	25 (CH_2Cl_2)	[242]
$[CpW(CO)_3]_2PbEt_2$	-128(CH_2Cl_2)	[242]

REFERENCES

[1] (a) F. Paneth and W. Hofeditz, Chem. Ber., 62 (1929) 1335; (b) F. Paneth and W. Lautsch, Chem. Ber., 64 (1931) 2702.

[2] D. Seyferth, Organometallics, 22 (2003) 2346 and 5154.

[3] (a) H. Shapiro and F.W. Frey, The Organic Compounds of Lead, Interscience Publishers, New York, 1968; (b) L.C. Willemsens, Organolead Chemistry, International Lead Zinc Research Organization ILZRO, New York, 1964; (c) L.C. Willemsens and G.J.M. van der Kerk, Investigations in the Field of Organolead Chemistry, International Lead Zinc Research Organization ILZRO, New York, 1965; (d) K.A. Kocheshkov, N.N. Zemlyanskii, N.I. Sheverdina and E.M. Panov, Metody Elemento-organicheskoi Khimii, Germanii, Olovo, Svinets, Izd. Nauka, Moskva 1968 [Methods of Organo-element Chemistry. Germanium, Tin, Lead – in Russian]; (e) Z. Rappoport (ed.), The Chemistry of Organic Germanium, Tin and Lead Compounds, Wiley-Interscience, New York, 2002; (f) Gmelin Handbook of Inorganic and Organometallic Chemistry, Organolead Compounds, F. Huber, ed., Springer Verlag, Berlin, Part 1 (1987), Part 2 (1990), Part 3 (1992).

[4] (a) W.P. Neumann and K. Kühlein, Adv. Organomet. Chem., 7 (1968) 242; (b) L.C. Willemsens, G.J.M. Van der Kerk, in Organometallic Compounds of the Group-IV Elements, A.G. MacDiarmid (ed.), M. Dekker, New York, 1968, Vol.1, Part II, p. 1911; (c) G. Bähr, E. Langer and A. Segnitz, in Methoden der Organischen Chemie (Houben-Weyl), Band XIII/7, G. Thieme Verlag, Stuttgart, 1975; (d) P.G. Harrison, in G. Wilkinson, F.G.A. Stone and E.W. Abel (eds.), Comprehensive Organometallic Chemistry, Pergamon Press, Oxford, 1982, Volume 2, p. 629; (e) I. Haiduc and J.J. Zuckerman, Basic Organometallic Chemistry, Walter de Gruyter, Berlin, New York, 1985, p. 173; (f) P.G. Harrison, in E.W. Abel, F.G.A. Stone and G. Wilkinson (eds.), Comprehensive Organometallic Chemistry II, Pergamon Press, Oxford, 1995, Volume 2, p. 305; (g) R.B. King, Encyclopedia of Inorganic Chemistry, 1995, p. 1955.

[5] (a) R.W. Leeper, L. Summers and H. Gilman, Chem. Rev., 54 (1954) 101; (b) D. DeVos and G. Wolters, Revs. Silicon, Germanium, Tin, Lead Compds., 4 (1980) 209; (c) M. Driess, H. Grützmann, Angew. Chem., Int. Ed. Engl. 35 (1996) 827; (d) P.P. Power, Chem. Rev., 99 (1999) 3463; (e) M. Weidenbruch, Eur. J. Inorg. Chem., (1999) 373; (e) N. Tokitoh and R. Okazaki, Coord. Chem. Revs., 210 (2002) 251; (f) K. Klinkhammer, Polyhedron, 21 (2002) 587; (g) M. Weidenbruch, J. Organomet. Chem., 646 (2002) 39; (h) M. Weidenbruch, Organometallics, 22 (2003) 4348.

[6] C. Löwig, J. Prakt. Chem. 60 (1853) 304; Liebigs Ann. Chem., 88 (1853) 318.

[7] H. Shapiro, Advan. Chem. Ser., 23 (1959) 290.

[8] F.W. Frey, P. Kobetz, G.C. Robinson and T.O. Sistrunk, J. Org. Chem., 26 (1961) 2950.

[9] K. Ziegler and H. Lehmkuhl, Angew. Chem., 67 (1955) 424.

[10] E. Guccione, Chem. Eng., 72 (1965) 102.

[11] (a) H. Gilman and J. Robinson, J. Am. Chem. Soc., 49 (1927) 2315; (b) D.E. Fenton and A.G. Massey, J. Inorg. Nucl. Chem., 27 (1965) 329.

[12] L.C. Willemsens and G.J.M. Van der Kerk, J. Organomet. Chem., 2 (1964) 260, 271.

[13] H. Gilman and R.G. Jones, J. Am. Chem. Soc., 72 (1950) 1760; H. Gilman and D.S. Maelstrom, J. Am. Chem. Soc., 72 (1950) 2953.

[14] (a) A.K. Brisdon, I.R. Crossley, R.G. Pritchard and J.E. Warren, Inorg. Chem., 41 (2002) 4748; (b) A.K. Brisdon, I.R. Crossley, R.G. Pritchard, G. Sadiq and J.E. Warren, Organometallics, 22 (2003) 5534; (c) A.K. Brisdon, I.R. Crossley, J.A. Greenall, R.G. Pritchard, J.E. Warren, J. Fluorine Chem., 125 (2004) 1099.

[15] T.H. Pearson, S.M. Blitzer and D.R. Carley, Advan. Chem. Ser., 23 (1959) 299.

[16] L.I. Zakharkin and O. Yu. Okhlobystin, Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk, (1959) 1942.
[17] E. Krause and G.G. Reissaus, Chem. Ber., 55 (1922) 888.
[18] H. Gilman, L. Summers and R.W. Leeper, J. Org. Chem., 17 (1952) 630.
[19] E.M. Dobratz, US Patent 2816123 (1970); Chem. Abstr., 52 (1958) 7344.
[20] (a) R.C. Juenge and S. Gray, J. Organomet. Chem., 10 (1967) 465; (b) G. Grüttner and E. Krause, Chem. Ber., 49 (1916) 2666; (c) K.C. Williams, J. Organomet. Chem., 19 (1969) 210.
[21] K.C. Williams, J. Organomet. Chem., 19 (1969) 210.
[22] H. Gilman and E.A. Zuech, J. Am. Chem. Soc. 82 (1960) 2522.
[23] H. Fleischer, S. Parsons and C.R. Pulham, Acta Crystallogr., E 59 (2003) m11.
[24] (a) K. Claborn, B. Kahr and W. Kaminsky, CrystEngComm, 4 (2002) 252; (b) V. Busetti, M. Mammi, A. Signor and A. Del Pra, Inorg. Chim. Acta, 1 (1967) 424.
[25] C. Schneider-Koglin, K. Behrends and M. Dräger, J. Organomet. Chem., 448 (1993) 29.
[26] C. Schneider-Koglin, B. Mathiasch and M. Dräger, J. Organomet. Chem., 469 (1994) 25.
[27] C. Schneider and M. Dräger, J. Organomet. Chem., 415 (1991) 349.
[28] T.M Klapötke, B. Krumm, M. Niemitz, K. Pohlborn and C.M. Rienacker, J. Fluorine Chem., 104 (2000) 129.
[29] T. Munguia, I.S. Pavel, R.N. Kapoor, F. Cervantes-Lee, L. Párkányi and K.H. Pannell, Can. J. Chem., 81 (2003) 1388.
[30] R. Villazana, H.K. Sharma, F. Cervantes-Lee and K.H. Pannell, Organometallics, 12 (1993) 4278.
[31] L.C. Willemsens, J. Organomet. Chem., 27 (1971) 45.
[32] L.C. Willemsens and G.J.M. van der Kerk, J. Organomet. Chem., 23 (1970) 471.
[33] M. Shimizu, T. Kurahashi, H. Kitagawa, K. Shimono and T. Hiyama, J. Organomet. Chem., 686 (2003) 286.
[34] A. M. Haiduc, and K. H. Pannell, Congreso Iberoamericano de Quimica Inorganica, 6th, Puebla, Mexico, Apr. 20-25 (1997) 251.
[35] (a) K. Lukin, J. Li, R. Gilardi and P.E. Eaton, Angew. Chem., 35 (1996) 866; (b) K.A. Lukin, J. Li, P.E. Eaton, N. Kanomata, J. Hain, E. Punzala and R. Gilardi, J. Am. Chem. Soc., 119 (1997) 9591; (c) K.A. Lukin, J. Li, P.E. Eaton and R. Gilardi, J. Org. Chem., 62 (1997) 8490.
[36] T. Müller, C. Bauch, M. Bolte and N. Auer, Chem. Eur. J., 9 (2003) 1746.
[37] T. Müller, C. Bauch, M. Ostermeier M. Bolte and N. Auner, J. Am. Chem. Soc., 125 (2003) 2158.
[38] M.A. Beswick, M.K. Davies, P.R. Raithby, A. Steiner and D.S. Wright, Organometallics, 16 (1997) 1109.
[39] F. Stabenow, W. Saak and M. Weidenbruch, Chem. Commun., (1999) 1131.
[40] (a) D.P. Arnold and P.R. Wells, J. Organomet. Chem. 111 (1976) 269; (b) S.M. Whittaker, F. Cervantes-Lee and K.H. Pannell, Inorg. Chem., 33 (1994) 6406.
[41] H. Preut and F. Huber, Z. Anorg. Allg. Chem., 419 (1976) 92.
[42] F. Stabenow, W. Saak and M. Weidenbruch, Chem. Commun., (1999) 2342.
[43] Y. Wang, B. Quillian, P. Wei, X.J. Yang and G.H. Robinson, Chem. Commun., (2004) 2224.
[44] W. Drenth, M.J. Jansen, G.J.M. Van der Kerk and J.A. Vliegenthart, J. Organomet. Chem., 2 (1964) 265.
[45] D.J. Davidson and M.F. Lappert, J. Chem. Soc., Chem. Commun., (1973) 317.

[46] (a) N. Kano, K. Shibata, N. Tokitoh and R. Okazaki, Organometallics, 18 (1999) 2999; (b) D.J. Davidson, D.H. Harris and M.F. Lappert, J. Chem. Soc., Dalton Trans., (1976) 2268; (c) M. Driess, R. Januschek, H. Pritzkow, S. Rell and U. Winkler, Angew. Chem., Int. Ed., 34 (1995) 1614; (d) J. Klett, K.W. Klinkhammer and M. Niemeyer, Chem. Eur. J., 5 (1999) 2531; (e) N. Tokitoh, N. Kano, K.Shibata and R. Okazaki, Organometallics, 14 (1995) 3121.
[47] N. Seidel, K. Jacob, A.A.H. van der Zeijden, H. Menge, K. Merzweiler and C. Wagner, Organometallics, 10 (2000) 1438.
[48] P.B. Hitchcock, M.F. Lappert and Z.Z. Wang, Chem. Commun., (1997) 1113.
[49] S. Wingerter, H. Gornitzka, R. Bertermann, S.K. Pandey, J. Rocha and D. Stalke, Organometallics, 19 (2000) 3890.
[50] (a) W.P. Leung, Z.X. Wang, H.W. Li and T.C.W. Mak, Angew. Chem., Int. Ed., 40 (2001) 2501; (b) W.P. Leung, Z.X. Wang, H.W. Li, Q.C. Yang and T.C.W. Mak, J. Am. Chem. Soc., 123 (2001) 123.
[51] W.P. Leung, W.H. Kwok, L.H. Weng, L.T.C. Law, Z.Y. Zhou and T.C.W. Mak, J. Chem. Soc., Dalton Trans., (1997) 4301.
[52] C. Eaborn, T. Ganicz, P.B. Hitchcock, J.D. Smith and S.E. Sözerli, Organometallics, 16 (1997) 5621.
[53] S. Hino, M. Brynda, A.D. Phillips and P.P. Power, Angew. Chem., Int. Ed., 43 (2004) 2655.
[54] K.W. Klinkhammer, T.F. Fässler and H. Grützmacher, Angew. Chem., Int. Ed., 37 (1998) 124.
[55] M. Stürmann, M. Weidenbruch, K.W. Klinkhammer, F. Lissner and H. Marsmann, Organometallics, 17 (1998) 4425.
[56] (a) G. Trinquier, J. Am. Chem. Soc., 112 (1990) 2130; (b) H. Jacobson and T. Ziegler, J. Am. Chem. Soc., 116 (1994) 3667.
[57] M. Stürmann, W. Saak, H. Marsmann and M. Weidenbruch, Angew. Chem., Int. Ed., 38 (1999) 187.
[58] M. Stürmann, W. Saak, M. Weidenbruch and K.W. Klinkhammer, Eur. J. Inorg. Chem., (1999) 579.
[59] F. Stabenow, W. Saak, H. Marsmann, M. Weidenbruch, J. Am. Chem. Soc., 125 (2003) 10172.
[60] (a) P.P. Power, Chem. Commun., (2003) 2091; (b) P.P. Power, Appl. Organomet. Chem., 19 (2005) 488; (c) L. Pu, B. Twamley and P.P. Power, J. Am. Chem. Soc., 122 (2000) 3524.
[61] E.O. Fischer and H. Gruber, Z. Anorg. Allg. Chem., 286 (1956) 237.
[62] (a) J.S. Overby, T.P. Hanusa and V.G. Young, Jr., Inorg. Chem., 37 (1998) 163; (b) C.A. Morrison, D.S. Wright and R.A. Layfield, J. Am. Chem. Soc., 124 (2002) 6775.
[63] R.A. Layfield, C.A. Morrison and D.S. Wright, J. Organomet. Chem., 650 (2002) 75.
[64] M.A. Beswick, C. Lopez-Casideo, M.A. Paver, P.R. Raitby, C.A. Russell, A. Steiner and D.S. Wright, J. Chem. Soc., Chem. Commun., (1997) 109.
[65] P. Jutzi and N. Burford, Chem. Rev., 99 (1999) 969.
[66] (a) M. Veith, M. Olbrich, W. Shihua and V. Huch, J. Chem. Soc., Dalton Trans., (1996) 161; (b) M. Veith, A. Rammo, S. Faber and B. Schillo, Pure Appl. Chem., 71 (1999) 401.
[67] J.L. Atwood, W.E. Hunter, A.H. Cowley, R.A. Jones and C.A. Stewart, Chem. Commun., (1981) 925.
[68] W.J. Evans, R.D. Clark, K.J. Forrestal and J.W. Ziller, Organometallics, 18 (1999) 2401.

[69] (a) S.P. Constantine, P.B. Hitchcock and G.A. Lawless, Organometallics, 15 (1996) 3905; (b) S.P. Constantine, H. Cox, P.B. Hitchcock and G.A. Lawless, Organometallics, 19 (2000) 317.
[70] D.J. Burkey, T.P. Hanusa and J.C. Huffman, Inorg. Chem., 39 (2000) 153, 1834.
[71] H. Schumann, C. Janiak, E. Hahn, C. Kolak, J. Loebel, M.D. Rausch, J.J. Zuckerman and M.J. Heeg, Chem. Ber., 119 (1986) 2856.
[72] R.M. Bellabarba, G.P. Clancy, P.T. Gomes, A.M. Martins, L.H. Rees and M.L.H. Green, J. Organomet. Chem., 640 (2001) 93.
[73] P.S. Tanner, J.S. Overby, M.M. Henein and T.P. Hanusa, Chem. Ber., 130 (1997) 155.
[74] R.E. Allan, M.A. Beswick, M.K. Davies, P.R. Raithby, A. Steiner, D.S. Wright, J. Organomet Chem., 550 (1998) 71.
[75] K.W. Hellmann, L.H. Gade, O. Gevert, P. Steinart and J.W. Lauher, Inorg. Chem., 34 (1995) 4069.
[76] (a) M.A. Beswick, N.L. Cromhout, C.N. Harmer, P.R. Raithby, C.A. Russell, J.S.B. Smith, A. Steiner and D.S. Wright, Chem. Commun., (1996) 1977; (b) D.R. Armstrong, M.A. Beswick, N.L. Cromhout, C.N. Harmer, D. Moncrieff, C.A. Russell, P.R. Raithby, A. Steiner, A.E.H. Wheatley and D.S. Wright, Organometallics, 17 (1998) 3176.
[77] A.K. Holliday, P.H. Makin and R.J. Puddephatt, J. Chem.. Soc., Dalton Trans., (1979) 228.
[78] M.A. Beswick, J.S. Palmer and D.S. Wright, Chem. Soc. Rev., 27 (1998) 225.
[79] D.R. Armstrong, M.J. Duer, M.G. Davidson, D. Moncrieff, C.A. Russell, C. Stourton, A. Steiner, D. Stalke and D.S. Wright, Organometallics, 16 (1997) 3340.
[80] R.A. Layfield, M. McPartlin and D.S. Wright, Organometallics, 22 (2003) 2528.
[81] N. Feeder, A.D. Hopkins, R.A. Layfield and D.S. Wright, J. Chem. Soc., Dalton Trans., (2000) 2247.
[82] M.A. Beswick, H. Gornitzka, J. Karcher, M.E.G. Mosquera, J.S. Palmer, P.R. Raithby, C.A. Russell, D. Stalke, A. Steiner and D.S. Wright, Organometallics, 18 (1999) 1148.
[83] (a) A.H. Cowley, J.N. Jones and C.L.B. Macdonald, Appl. Organomet. Chem., 19 (2005) 578; (b) J.N. Jones, J.A. Moore, A.H. Cowley and C.L.B. Macdonald, J. Chem. Soc., Dalton Trans., (2005) 3846.
[84] G.M. De Lima, D.J. Duncalf and S.P. Constantine, Main Group Met. Chem., 23 (2000) 3307.
[85] J.S. Overby, T.P. Hanusa and P.D. Boyle, Angew. Chem., Int. Ed. Engl., 36 (1997) 2378.
[86] N. Kuhn, G. Henkel and S. Stubenrauch, Angew. Chem., Int. Ed., 31 (1992) 778.
[87] G.E. Herberich, X. Zheng, J. Rosenplänter and U. Englert, Organometallics, 18 (1999) 4747.
[88] X. Zheng and G.E. Herberich, Eur. J. Inorg. Chem., (2001) 3013.
[89] K. Forissier, L. Ricard, D. Carmichael and F. Mathey, Chem. Commun., (1999) 1273.
[90] J.J. Durkin, M.D. Francis, P.B. Hitchcock, C. Jones and J.F. Nixon, J. Chem. Soc., Dalton Trans., (1999) 4057.
[91] F. D. Mathew and P.B. Hitchcock, Organometallics, 22 (2003) 2891.
[92] W. Frank and F.G. Wittmer, Chem. Ber.,130 (1997) 1731.
[93] A.G. Gash, P.F. Rodesiler and E.L. Amma, Inorg. Chem., 13 (1974) 2429.
[94] H. Basch, Inorg. Chim. Acta, 242 (1996) 191.
[95] W.P. Neumann and K. Kühlein, Angew. Chem., Int. Ed., 4 (1965) 784.
[96] R. Duffy, J. Feney and A.K. Holliday, J. Chem.. Soc., (1962) 1144.
[97] W.E. Becker and S.E. Cook, J. Am. Chem. Soc., 82 (1960) 6264.
[98] (a) G. Grütner and E. Krause, Chem. Ber., 49 (1916) 1415; (b) G. Grütner, Chem. Ber., 51 (1918) 1298; (c) H. Gilman and C. Bailey, J. Am. Chem. Soc., 61 (1939) 731.

[99] (a) E. Krause and M. Schmitz, Chem. Ber., 52 (1922) 888; (b) H.J. Emeléus and P.R. Evans, J. Chem. Soc., (1964) 511.
[100] (a) B.C. Saunders and G.J. Stacey, J. Chem. Soc., (1949) 919; (b) R.Heap and B.C. Saunders, J. Chem. Soc., (1949) 2983; (c) R. Heap, B.C. Saunders, G.J. Stacey, J. Chem. Soc., (1951) 658; (d) G. Calingaert, F.J. Dykstra and H. Shapiro, J. Am. Chem. Soc., 67 (1945) 190; (e) E. Lieber, C.N. Rao and F.M. Keane, J. Inorg. Nucl. Chem., 25 (1963) 631; (f) E.C. Junge and S.E. Cook, J. Am. Chem. Soc., 81 (1959) 3578.
[101] R. Gelius, Z. Anorg. Allg. Chem., 334 (1964) 72.
[102] B.C. Pant and W.E. Davidsohn, J. Organomet. Chem., 39 (1972) 295.
[103] (a) E. Krause and E. Pohland, Chem. Ber., 55 (1922) 1282; (b) E. Kunze and F. Huber, J. Organomet. Chem., 57 (1973) 345.
[104] M. Lesbre, Hebd. CR Acad.Sci., 210 (1940) 535.
[105] G. Chobert and M. Devaud, J. Organomet. Chem., 153 (1978) C 23.
[106] M. Kobayashi, S. Latour and J.D. Wuest, Organometallics, 10 (1991) 2908.
[107] M. Kaupp and P.v. R. Schleyer, J. Am. Chem. Soc., 115 (1993) 1061.
[108] L. Pu, B.Twamley and P.P. Power, Organometallics, 19 (2000) 2874.
[109] L. Pu, P.P. Power, I. Boltes and R. Herbst-Irmer, Organometallics, 19 (2000) 352.
[110] A.C. Filippou, H. Rohde and G. Schnackenburg, Angew. Chem., Int. Ed., 43 (2004) 2243.
[111] A.C. Filippou, N. Weidemann, G. Schnackenburg, H. Rohde and A.I. Phillippopoulos, Angew. Chem., Int. Ed., 43 (2004) 6512.
[112] S. Hino, M. Olmstead, A.D. Phillips, R.J. Wright and P.P. Power, Inorg. Chem., 43 (2004) 7346.
[113] C. Eaborn, P.B. Hitchcock, J.D. Smith and S.E. Sözerli, Organometallics, 16 (1997) 5653.
[114] P.B. Hitchcock, M.F. Lappert and M. Layh, Inorg. Chim. Acta, 269 (1998) 181.
[115] A.K. Holliday, P.H. Makin and R.J. Puddephatt, J. Chem. Soc., Dalton Trans., (1976) 435.
[116] P. Jutzi, R. Dickbreder and H. Nöth, Chem. Ber., 122 (1989) 865.
[117] P. Jutzi and R. Dickbreder, J. Organomet. Chem., 373 (1989) 301.
[118] A.G. Gash, P.F. Rodesiler and E.L. Amma, Inorg. Chem. 13 (1974) 2429.
[119] (a) N.A. Matwiyoff and R.S. Drago, J. Organomet. Chem., 3 (1965) 393; (b) R. West, R.H. Baney and D.L. Powell, J. Am. Chem. Soc., 82 (1960) 6269.
[120] G. Bähr, Z. Anorg. Allg. Chem., 253 (1947) 330.
[121] R. Heap, B.C. Saunders and G.J. Stacey, J. Chem. Soc., (1951) 658.
[122] H. Lindemann and F. Huber, Z. Anorg. Allg. Chem., 394 (1972) 101.
[123] H. Poleschner, M. Heydenreich and U. Schilde, Eur. J. Inorg. Chem., (2000) 1307.
[124] (a) M.M. Koton, Zhur. Obsh. Khim., 11 (1941) 376; (b) J.R. Kalman, J. Morgan, J.T. Pinhey and S. Sternhell, Tetrahedron, 55 (1999) 3615.
[125] E.M. Panov and K.A. Kocheshkov, Dokl. Akad. Nauk SSSR, 85 (1952) 1037.
[126] (a) K.A. Kocheshkov and E.M. Panov, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, (1955) 711; (b) V.I. Lodochnikova, E.M. Panov and K.A. Kocheshkov, Zhur. Obshch. Khim., 33 (1963) 1199; (c) V.I. Lodochnikova, E.M. Panov and K.A. Kocheshkov, Zhur. Obshch. Khim., 34 (1964) 4022.
[127] L.C. Willemsens and G.J.M. van der Kerk, J. Organomet. Chem., 13 (1968) 357.
[128] F. Huber, H. Preut and M. Schürmann, J. Organomet. Chem., 441 (1992) 227.
[129] (a) A.G. Davies and R.J. Puddephatt, J. Chem. Soc. C, (1967) 2663; (b) R.J. Puddephatt and G.H. Thistlewaite, J. Chem. Soc., Dalton Trans., (1972) 570; (c) R. Honigschmidt-Grossich and E. Amberger, Chem. Ber., 102 (1969) 589.
[130] M. Veith, C. Mathur, S. Mathur and V. Huch, Organometallics, 16 (1997) 1292.

[131] S.R. Bahr and P. Boudjouk, Inorg. Chem., 31 (1992) 4015.
[132] B.M. Schmidt and M. Dräger, J. Organomet. Chem., 399 (1990) 63.
[133] H. Stenger, B.M. Schmidt and M. Dräger, Organometallics. 14 (1995) 4374.
[134] O.R. Flöck and M. Dräger, Organometallics, 12 (1993) 4623.
[135] (a) N. Kano, K. Shibata, N.Tokitoh, R. Okazaki, Organometallics, 18 (1999) 2999; (b) N. Tokitoh, N. Kano, K. Shibata and R. Okazaki, Organometallics, 14 (1995) 3121.
[136] N. Kano, N. Tokitoh and R. Okazaki, Organometallics, 16 (1997) 4237.
[137] U. Herzog and G. Rheinwald, J. Organomet. Chem., 648 (2002) 220.
[138] G.D. Andretti, G. Bocelli, G. Calestani and P. Sgarabotto, J. Organomet. Chem., 273 (1984) 31.
[139] E.M. Holt, F.A.K. Nasser, A. Wilson, Jr., and J.J. Zukermann, Organometallics, 4(1985) 2073.
[140] K. Tani, S. Kato, T. Kanda and S. Inagaki, Organic Letters, 3 (2001) 655.
[141] S. Kato, K. Tani, N. Kitaoka, K. Yamada and H. Mifune, J. Organomet. Chem., 611 (2000) 190.
[142] M.J. Begley, C. Gaffney, P.G. Harrison and A. Steel, J. Organomet. Chem., 289 (1985) 281.
[143] S.N. Olafsson, T.N. Petersen and P. Andersen, Acta Chem. Scand., 50 (1996) 745.
[144] R.A. Varga, C. Silvestru and I. Haiduc, Syn. React. Inorg. Metal-org. Chem., 30 (2000) 485.
[145] L. Silaghi-Dumitrescu and I. Haiduc, J. Organomet. Chem., 259 (1983) 65.
[146] J.M.S. Skakle, J.L. Wardell and S.M.S.V. Wardell, Acta Crystallogr., 57 C (2001) 787.
[147] S.M.S.V. Doidge-Harrison, J.T.S. Irvine and G.M. Spencer, Polyhedron, 15 (1996) 1807.
[148] F. Grätz, F.Huber, A. Silvestri, G. Alonzo and R. Barbieri, J.Organomet. Chem., 290 (1985) 41.
[149] M. Dräger and N. Kleiner, Angew. Chem., Int. Ed. Engl., 19 (1980) 923.
[150] M. Dräger and N. Kleiner, Z. Anorg. Allg. Chem., 522 (1985) 48.
[151] T.M. Klapötke, B. Krumm and P. Mayer, Z. Naturforsch., 59b (2004) 547.
[152] S. Kato, H. Ishihara, K. Ibi, H. Kageyama and T. Murai, J. Organomet. Chem., 386 (1990) 313.
[153] C.W. Liu, T.S. Lobana, J.L. Xiao, H.Y. Liu, B.J. Liaw, C.M. Hung and Z. Lin, Organometallics, 24 (2005) 4072.
[154] K. Tano, R. Yamada, T. Kanda, M. Suzuki, S. Kato, M. Shinzi and T. Murai, Organometallics, 21 (2002) 1487.
[155] (a) H.J. Götze and W. Garbe, Z. Anorg. Allg. Chem., 454 (1979) 99; (b) H.J. Götze and W. Garbe, Spectrochim. Acta, A 38 (1982) 153.
[156] (a) W.P. Neumann and K. Kühlein, Tetrahedron Lett., (1966) 3419; (b) O. J. Scherer and M. Schmidt, J. Organomet. Chem., 1 (1964) 490; (c) I. Ruidisch and M. Schmidt, Angew. Chem., 76 (1969) 686.
[157] C. Stanciu, S.S. Hino, M. Stender, A.F. Richards, M.M. Olmsted and P.P. Power, Inorg. Chem., 44 (2005) 2774.
[158] (a) A. Blaschette, T. Hamann, A. Michalides and P.G. Jones, J. Organomet. Chem., 456 (1993) 49; (b) O. Hiemisch, D. Henschel, A. Blaschette and P.G. Jones, Z. Anorg. Allg. Chem., 623 (1997) 324.
[159] (a) H. Schumann and R. Fischer, J. Chem. Res., (1977) S 272; (b) H. Schumann, P. Schwabe and M. Schmidt, J. Organomet. Chem., 1 (1964) 366.
[160] D. Weber, C. Mujica and H.G. von Schnering, Angew. Chem., Int. Ed. Engl., 21 (1982) 863.

[161] (a) H. Schumann and M. Schmidt, Inorg. Nucl. Chem.. Lett., 1 (1965) 1; (b) H. Schumann, A. Roth, O. Stelzer and M. Schmidt, Inorg. Nucl. Chem. Lett., 2 (1966) 311.
[162] S.P. Mallela and R.A. Geanagel, Inorg. Chem., 32 (1993) 602.
[163] U. Englich, E. Hengge, U. Hermann, C. Marschner, K. Ruhland-Senge and F. Uhlig, J. Organomet. Chem., 605 (2000) 22.
[164] C. Tamborski, F.E. Ford, W.L.Lehn, G.J. Moore and E.J. Soloski, J. Org. Chem., 27 (1962) 619.
[165] (a) N. Kleiner and M. Dräger, J. Organomet. Chem., 270 (1984) 151; (b) H.J. Koglin, K. Behrendt and M. Dräger, Organometallics, 13 (1994) 2733.
[166] L.C. Willemsens and G.J.M. van der Kerk, J. Organomet. Chem., 15 (1968) 117.
[167] D.R. Armstrong, M.G. Davidson, D. Moncrief, D. Stalke and D.S. Wright, Chem. Commun., (1992) 1413.
[168] (a) L.C. Willemsen and G.J.M. van der Kerk, J. Organomet. Chem., 21 (1970) 123; (b) K.C. Williams, J. Organomet. Chem., 22 (1970) 139.
[169] K.C. Williams, J. Organomet. Chem., 23 (1970) 465.
[170] H.K. Sharma, I. Haiduc and K.H. Pannell, in "The Chemistry of Organic Germanium, Tin and Lead Compounds", ed. Z. Rappoport, J. Wiley & Sons, Ltd., New York, 2002, Vol. 2, p. 1241.
[171] S. Seebald, G. Kickelbick, F. Möller and U. Schubert, Chem. Ber., 129 (1996) 1131.
[172] (a) F. Calderazzo, G. Pampaloni, G. Pelizzi and F. Vitali, Polyhedron, 7 (1988) 2039; (b) R. Talay and H. Rehder, J. Organomet. Chem., 262 (1984) 25.
[173] R.D. Gorsich, J. Am. Chem. Soc., 84 (1962) 2486.
[174] W. Jetz, P.B. Simons, J.A.J. Thompson and W.A.G. Graham, Inorg. Chem., 5 (1966) 2217.
[175] K.H. Pannell, J.M. Rozell, S. Cortez and R. Kapoor, Organometallics, 9 (1990) 1322.
[176] H.R.H. Patil and W.A.G. Graham, Inorg. Chem., 5 (1966) 1401.
[177] J.M. Geller, J.H. Wosnik, D.F.R. Gilson, F.G. Morin and F. Belanger-Gariepy, Can. J. Chem., 80 (2002) 813.
[178] K.H. Pannell and R. Kapoor, J. Organomet. Chem., 214 (1981) 47.
[179] K.H. Pannell, J. Organomet. Chem., 198 (1980) 37.
[180] M. Herberhold, V. Tröbs, W. Milius and B. Wrackmeyer, Z. Naturforsch., 49b (1994) 1781.
[181] (a) B. Crociani, M. Nicolini, D.A. Clemente and G. Bandoli, J. Organomet. Chem., 49 (1973) 249; (b) G. Deganello, G. Carturan and U. Bellucco, J. Chem. Soc., A (1968) 2873.
[182] T. Habereder and H. Nöth, Appl. Organomet. Chem., 17 (2003) 525.
[183] N. Seidel, K. Jacob and A.K. Fischer, Organometallics, 20 (2001) 578.
[184] H.J. Haupt, W. Schubert and F. Huber, J. Organomet. Chem., 54 (1973) 231.
[185] R.D. Gorsich, J. Am. Chem. Soc., 84 (1962) 2486.
[186] C. Campbell and L.J. Farrugia, Acta Crystallogr., C 45 (1989) 1817.
[187] N.C. Burton, C.J. Cardin, D.J. Cardin, B. Twamley and Y. Zubavichus, Organometallics, 14 (1995) 5708.
[188] R.A. Howe and J.L. Wardell, Acta Crystallog., E 57 (2001) m256.
[189] D. Weber, S.H. Hausner, A. Eisengräber-Pabst, S. Yun, J.A. Krause-Bauer and H. Zimmer, Inorg. Chim. Acta, 357 (2004) 125.
[190] G. Pilloni and F. Magno, Inorg. Chim. Acta, 4 (1970) 105.
[191] N.A. Matwiyoff and R.S. Drago, Inorg. Chem., 3 (1964) 337.
[192] H.J. Eppley, J.L. Eally, C.H. Yoder, J.N. Spencer and A.L. Rheingold, J. Organomet. Chem., 431 (1992) 133.

[193] K.M. Lo, J.Y. Niu and J.P. Wang, Chin. J. Inorg. Chem., 16 (2000) 763.
[194] S.W. Ng, Acta Crystallogr., C 51 (1995) 2042.
[195] A.V. Yatsenko, L.A. Aslanov and H. Schenk, Polyhedron, 14 (1995) 2371.
[196] (a) K. Jurkschat, K. Peveling and M. Schürmann, Eur. J. Inorg. Chem., (2003) 3563; (b) K. Peveling, M. Schürmann and K. Jurkschat, Z. Anorg. Allg. Chem., 628 (2002) 2435.
[197] S.N. Olafsson, C. Flensburg and P. Andersen, J. Chem. Soc., Dalton Trans., (2000) 4360.
[198] J.S. Casas, E.E. Castellano, J. Ellena, M.S. Garcia-Tasende, A.Sanchez, J. Sordo and M.J. Vidarte, Main Group Met. Chem., 24 (2001) 457.
[199] A. Zickgraf, M. Beuter, U. Kolb, M. Dräger, R. Tozer, D. Dakternieks and K. Jurkschat, Inorg. Chim. Acta, 275/276 (1998) 203.
[200] H.O. van der Kooi, W.H. den Brinker and A.J. de Kok, Acta Crystallogr., C 41 (1985) 869.
[201] W.L. Jia, Q.D. Liu, R. Wang and S. Wang, Organometallics, 22 (2003) 4070.
[202] (a) J.-M. Lehn, Angew. Chem., Int. Ed. Engl., 27 (1988) 89; (b) J.-M. Lehn, Supramolecular Chemistry. Concepts and Perspectives, VCH, Weinheim, 1995.
[203] I. Haiduc and F. Edelmann, Supramolecular Organometallic Chemistry, Wiley-VCH, Weinheim, New York, 1999.
[204] C. Glidewell and D.C. Liles, Acta Crystallogr., B 34 (1978) 129.
[205] G.M. Sheldrick and R. Taylor, Acta Crystallogr., B 31 (1975) 2740.
[206] H. Preut, P. Röhm and F. Huber, Acta Crystallogr., C 42 (1986) 657.
[207] M. Schürmann and F. Huber, J. Organomet. Chem., 530 (1997) 121.
[208] R.A. Varga, J.E. Drake and C. Silvestru, J. Organomet. Chem., 675 (2003) 48.
[209] A.F. Shihada and F. Weller, Z. Anorg. Allg. Chem., 627 (2001) 638.
[210] M.C. Henry, Inorg. Chem., 1 (1962) 917.
[211] (a) R.J.H. Clark, A.G. Davies and R.J. Puddephatt, J. Am. Chem. Soc., 90 (1968) 6923; (b) R.J.H. Clark, A.G. Davies and R.J. Puddephatt, Inorg. Chem., 8 (1969) 457.
[212] R. Hillwig, F. Kunkel, K. Harms, B. Neumüller and K. Dehnicke, Z. Naturforsch., 52b (1997) 149.
[213] D. Zhang, S.Q. Dou and A. Weiss, Z. Naturforsch., 46a (1991) 337.
[214] H. Preut and F. Huber, Z. Anorg. Allg. Chem., 435 (1977) 234.
[215] U. Fahrenkampf, M. Schürmann and F. Huber, Acta Crystallogr., C 50 (1994) 1252.
[216] H.K. Sharma, R.J. Villazana, F. Cervantes-Lee, L. Párkányi and K.H. Pannell, Phosphorus, Sulfur, Silicon, 87 (1994) 257.
[217] M. Mammi, V. Busetti, A. Del Pra, Inorg. Chim. Acta, 1 (1967) 419.
[218] J.H. Aupers, P.J. Cox, S.M.S.V. Doidge-Harrison, R.A. Howie, J.N. Low and J.L. Wardell, Main Group Chem., 3 (1999) 23.
[219] F.T. Edelmann, I. Haiduc, C. Silvestru, H.G. Schmidt and M. N Noltemeyer, Polyhedron, 17 (1998) 2043.
[220] (a) R. Allmann, A. Waskowska, R. Hochfeld and J. Lorberth J. Organomet. Chem., 198 (1980) 155; (b) J. Müller, U. Müller, A. Loss, J. Lorberth, H. Donath and W. Massa, Z. Naturforsch., 40b (1985) 1320.
[221] Y.M. Chow and D. Britton, Acta Crystallogr., B 27 (1971) 856.
[222] N.G. Furmanova, Yu.T. Struchkov, D.N. Kravtsov and E.M. Rokhlina, Zhur. Strukt. Khim., 20 (1979) 1047.
[223] N. Bertazzi, G. Alonzo, A. Silvestri and G. Consiglio, J. Organomet. Chem., 37 (1972) 281.

[224] (a) U. Behrens, A.K. Brimah, T.M. Soliman, R.D. Fischer, D.C. Apperley, N.A. Davies and R.K. Harris, Organometallics, 11 (1992) 1718; (b) A.M.A. Ibrahim, S.E.H. Etaiw and T.M. Soliman, J. Organomet. Chem., 430 (1992) 87; (c) A.M.A. Ibrahim, T.M. Soliman, S.E.H. Etaiw and R.D. Fischer, J. Organomet. Chem., 468 (1994) 93; (d) A.K. Brimah, P. Schwarz, R.D. Fischer, N.A. Davies and R.K. Harris, J. Organomet. Chem., 568 (1998) 1; (e) P. Schwarz, E. Siebel, R.D. Fischer, N.A. Davies, D.C. Apperley and R.K. Harris, Chemistry-Eur. J., 4 (1998) 919; (f) A.M.A. Ibrahim, J. Mater. Chem., 8 (1998) 841.

[225] J.S. Casas, E.E. Castellano, J. Ellena, M.S. Garcia-Tasende, A. Sanchez, J. Sordo and M.J. Vidarte, Inorg. Chem., 42 (2003) 2584.

[226] A.V. Yatsenko, H. Schenk and L.A. Aslanov, J. Organomet. Chem., 474 (1994) 107.

[227] (a) A.W.P. Jarvie, Sci. Total Environm., 73 (1988) 121; (b) R. Lobiński, Analyst, 120 (1995) 615.

[228] S.N. Bhattacharya and P. Raj, M. Singh, Indian J. Chem., A 17 (1979) 355.

[229] W.J. Kinart, I. Tylak and C.M. Kinart, J. Chem. Res., S 1 (1999) 46.

[230] M. Le Quan, G. Guillerm, Compt. Rend. Acad. Sci., Ser. C, Sci. Chimiques, 268 (1969) 1001.

[231] D. Seyferth, D. Son and S. Shah, Organometallics, 13 (1994) 2105.

[232] (a) J.T. Pinhey, Pure Appl. Chem., 68(1996) 819; (b) J.T. Pinhey, in Comprehensive Organometallic Chemistry II, E.W. Abel, F.G.A. Stone and G. Wilkinson, eds., Pergamon Press, Oxford, 1995, vol. 11, Chapter 11.

[233] (a) C.M. H. Raju, P.N. Laksmi, C. Sinivas, G.O. Reddy, P.V.R. Acharyulu, Synth. Commun., 35 (2005) 209; (b) S.K. Kang, H.C. Ryu and S.H. Lee, Synth. Commun., 31 (2001) 1059.

[234] B. Wrackmeyer, Annu. Rep. NMR Spectrosc., 47 (2002) 1.

[235] R.S. Simons, L.H. Pu, M.M. Olmstead and P.P. Power, Organometallics, 16 (1997) 1920.

[236] S. Brooker, J.–K. Buijink, F.T. Elderman, Organometallics, 10 (1991) 25.

[237] M.J. Cooper, A.K. Holiday, P.H. Makin and R.J. Puddephatt, J. Organomet. Chem., 65 (1974) 377.

[238] J. D. Kennedy, W. McFarlane and G.S. Pyne, J. Chem. Soc. Dalton Trans., (1977) 2332.

[239] T.N. Mitchell, J. Gmehling and F. Huber, J. Chem. Soc., Dalton Trans., (1978) 960.

[240] H.–J. Koglin, K. Behrends and M. Dräger, Organometallics, 13 (1994) 2733.

[241] J.D. Kennedy, W. McFarlane and B. Wrackmeyer, Inorg. Chem., 15 (1976) 1299.

[242] M.M. Kubicki, J-V. Le Gall, R. Kergoat and L.C. Gomes de Lima, Can. J. Chem., 65 (1987) 1292.

Lead - J.S. Casas and J. Sordo (Editors)

Chapter 4

Environmental occurrence, health effects and management of lead poisoning

Swaran J.S. Flora[a], Govinder Flora[b] and Geetu Saxena[a]

[a]Division of Pharmacology and Toxicology, Defence Research and Development Establishment, Jhansi Road, Gwalior-474002, India

[b]Department of Neurological Surgery, The Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, 1095 NW 14th Terrace (R-48), Miami, FL 33136, USA

1. INTRODUCTION

Lead is a metal of antiquity and is detectable in practically all phases of the inert environment and in all biological systems, having widespread industrial applications. Lead has been mined and used in industry and in household products for centuries. Lead's atomic number may not be 1 (it is 82) but it ranks near the top when comes to industrial uses. The unique properties of lead-softness, malleability, low melting point, and resistance to corrosion make it one of the most widely used metals. Defused lead is produced from both primary and secondary sources. Primary lead is that produced from mined ores, while secondary lead is derived from recycled materials such as battery and lead pipes.

The dangers of lead toxicity, the clinical manifestations of which are termed '*plumbism*', have been known since ancient times. Significant exposure to lead is an environmental threat to optimal health and to physical development in young children that affects all socio-economic groups [1]. Tetraethyl lead and tetra methyl lead are used extensively as fuel additives. Both are volatile and poorly soluble in water. Trialkyl lead compounds are formed in the environment by the breakdown of tetra alkyl lead. These trialkyl compounds are less volatile and more readily soluble in water. Lead is widely distributed in nature. It is usually associated with other metals, particularly silver and zinc. Although, mined and used for centuries, galena still remains the principal source of lead today. Lead, a ubiquitous environmental toxin induces a broad range of physiological, biochemical and behavioural dysfunction. Lead poisoning is thus an environmental disease, but it's also a disease of lifestyle. Lead is known to affect the structure and function of various organs and tissues. The present

chapter provides a comprehensive account of environmental lead exposure, biochemical and toxic effects of lead, diagnosis, recent development in the preventive and therapeutic measures etc. in human and in experimental animals.

2. LEAD IN THE ENVIRONMENT

2.1. Occurrence

The level of lead in the earth's crust is about 20mg/kg. In the environment it may be derived from either natural or anthropogenic sources. The human species evolved in an essentially lead-free environment. Most of the lead present was buried in subsurface deposits composed of a relatively inert (insoluble) form. As a consequence humans and other living species have no known use for lead. The amount of lead on the earth's crust is larger than might be predicted. One reason is that it was concentrated during the earth forming process and a second is that it is the "sink" for radioactive decay of uranium and thorium. Because lead has several parent atoms it has variable mass which creates a fingerprint for various ore bodies. A lead sulphide containing ore found at the surface of the earth's crust undergoes weathering to the mineral $PbSO_4$, or *anglesite*. Further weathering may result in *cerussite*, $PbCO_3$. Lead and iron sulphide ore at the interface with unweathered ore, high in clay is known as *jarosite*. Typically a lead ore body consisted of surface oxidized ores: cerussite ($PbCO_3$), anglesite ($PbSO_4$), litharge (αPbO), and massicot (βPbO) below which lay plumbojarosite [$PbFe_6(SO_4)_2(OH)_{12}$], then galena (PbS). Most lead in ore bodies is in the form of galena or cerrusite, both of which are attractive minerals. Lead has four common isotopes: ^{204}Pb, ^{205}Pb, ^{207}Pb and ^{208}Pb. The last three forms of lead results from the radioactive decay of thorium and two different isotopes of uranium. Lead is found with silver and due to this reason it is extensively mined. Lead is also found in conjunction with many other trace elements, especially with antimony [2].

2.2. Sources of lead in our environment

Although lead is ubiquitous in the environment of industrialized nations, the contribution of natural sources of lead to concentrations in the environment is low compared to the contribution from human activities [3]. Through human activities such as mining, smelting, refining, manufacturing, and recycling, lead finds its way into the air, water, and surface soil. Lead-containing manufactured products (gasoline, paint, printing inks, lead water pipes, lead-glazed pottery, lead-soldered cans, battery casings, etc.) also contribute to the lead burden. Lead in contaminated soil and dust can find its way into the food and water supply.

The source of greatest concern is old housing, specifically houses once painted with products containing lead as a pigment. The chips and dust from peeling or cracking leaded paint remain highly toxic. Sanding, scraping or heating painted doors, windows, stairs or fences can release leaded dust into the

air, where children and adults may breathe it in. Even vacuuming, sweeping, or walking can circulate the dust, which eventually gathers on the floor where it is accessible to infants and toddlers engaging in hand-to-mouth activity [4]. Lead-based dust from deteriorating paint can also be present in the soil around old homes.

A second major source of lead exposure comes from soil contaminated by lead residues from leaded gasoline and industrial processes. For many years, lead smelters, battery plants, and automobiles released dangerous doses of lead-infused emissions into the air and soil. Traces of lead from these sources remain in the soil, particularly in urban areas [5].

Though the use of lead has been curtailed in many household products, the metal can still end up in food and water, mostly from residual sources. Food may be prepared with leaded utensils or stored in leaded pots and ceramics. Drinking water may be transported through old pipes soldered with lead.

2.2.1 Lead based paint

Due to lead's unique properties, it has been used as a pigment and drying agent in primers, paints and enamels, inks, oils, resins and other surface coatings for centuries. Throughout the 1940's and 1950's lead-based paint was in widespread use. It continued to be used in lower concentrations until the mid-1970's [6]. Although lead-containing paint was banned for residential use but paint on older buildings is the most frequent source of lead exposure in young children [7]. Lead has been used to make petroleum products, glass panels, crafts, batteries, jewelry, pencils, colored newsprints, etc. However, high lead content is estimated to be in 74 percent of all housing built before 1980. Those housing units containing deteriorating lead-based paints are the major concern. Of even greater concern are these homes that have young children as occupants [8].

Lead paint exposure accounts for as much as 90% of childhood lead poisoning. While generally considered an inner city problem, it is, in fact, not so. Lead paint not only directly poisons individuals, but contaminates soils and other surfaces which can also be the cause of poisoning [9].

Lead from different sources such as from lead-paint, gasoline, and solder may enter the body through air, food, water, dust and soil. Lead-based paint is still available for industrial, military and marine use and occasionally ends up being used in homes. Pica, a craving for unnatural food, is one way children are exposed to lead when they eat tiny pieces of peeling or chipping lead-based paint. More commonly, children ingest dust and soil contaminated with lead from paint that re-enter that flakes or chalks as it ages. Lead-contaminated house dust can settle on floors, walls, and furniture. Settled lead dust can re-enter the air through cleaning, such as vacuuming or sweeping, or by movement of people throughout the house. Lead-contaminated house dust, ingested via normal repetitive hand-to-mouth activity, is now recognized as a major contributor to

lead poisoning in children. Adults can also be exposed to lead in the same ways [10].

The risk of lead poisoning is related to both the presence and the condition of the paint. The risks of lead poisoning are greater when lead-paint has deteriorated or when lead-based paint (even intact paint) is located on surfaces accessible to children [11]. Lead allowed in paint is still potentially a problem because at all levels of exposure, lead poisoning causes severe adverse health effects in both children and adults, affecting their ability to learn and thrive, their productivity, and their global competitiveness [12].

2.2.2 Food, drinking water and air

2.2.2.1 Food

Lead in foods may be derived from the environment in which the food is grown or from food processing. Agricultural crops grown near heavily traveled roads or industrial sources of lead can have significant concentrations because of airborne lead deposited on them or in the soil. Among cases of lead poisoning cited in the literature, lead from ceramic glazed storage vessels, leached out by acid foods, is the most frequently-reported source of high lead concentrations in foods [13].

Lead contamination in food could be due to following ways:

- Soil, pesticide or zinc fertilizer containing lead may be taken up into a root, plant or deposited on leafy plants. Lead emissions from cars or industry may be deposited on plants grown in home or market gardens near main roads.

- Canned foods are a source of lead which is leached from the solder in the seams of the cans. However, exposure from this source can be reduced by the use of seamless cans [14]. Foods or beverages, particularly acidic foods such as pineapples, pickles and tomatoes may be packed in cans with lead solder side seams or processed by equipment containing lead soldering. Foods or beverages may be stored, cooked, reheated or served in lead glazed ceramics or porcelain, cook pots tinned with a lead-tin mixture, brass with leaching of lead, or leaded crystal or glass. Spices and food coloring may also be contaminated with lead from petrol emissions, lead pigments or painted storage containers.

- Some home remedies also contain lead compounds that can be the cause of lead poisoning. As many as 5% of children have high enough lead intake through water and food to cause health risks [15].

2.2.2.2 Drinking water

The major source of lead contamination of drinking water is the distribution system itself. Where lead water pipes or lead-lined cisterns are used, lead may contaminate the water supply and contribute to increased blood levels in children who consume the water [16]. Lead pipes for conveying drinking water has been used for centuries because of its flexibility, durability and long life. Water used to prepare infant formula is always a significant source of lead for infants if it contains high lead levels [17]. Water from lead-soldered water tanks or run-off systems from roofing with lead-based paint also pose a risk. Especially in areas near mining and smelting sites where dust and emissions could add to the problem.

Contamination of lead in groundwater originates from the dissolution of lead from soil and earth crust. Lead particulate from the combustion of leaded gasoline, fossil and ore smelting can contaminate local surface water by surface runoff. Lead itself has minor content in the earth crust. A widely distribution of lead in sedimentary rocks and soils are reported an average lead content of 10 mg in 1 kg (10 ppm) soil usually found in upper ground soil and lead in a range of 7 to 12.5 ppm is found in sedimentary rock [5]. The solubility of lead controls the lead dissolution into surrounding water. Absorption by soil and by humus can further limit the lead concentrations in surface waters and groundwater.

Lead in drinking water is probably absorbed more completely than food. Adult absorb 35 to 50% of the lead they drink and the absorption rate for children may be greater than 50%.

2.2.2.3 Air

Lead in the air comes from a variety of sources. One of the largest contributors has been from leaded gasoline. Millions of tons of lead were added to gas before use was limited by EPA regulations restricting the use of lead in gasoline. Much of this lead is still present in the environment as lead in soils and lead in dust. The atmospheric levels of lead depend on geographical location, with major differences in lead in the atmosphere in urban and remote areas of the world. The highest concentrations are observed near sources of lead such as smelters. Levels range from 0.000076 $\mu g/m^3$ in remote areas to up to 10 $\mu g/m^3$ in areas near smelters [18]. The major contribution to ambient lead originates from the combustion of lead containing fuels used in internal combustion engines, the combustion of lead containing coal or fuel oil, the activities in lead mining and refining. Anthropogenic sources of atmospheric particulate include those arising from the burning of coal (fly ash) and petroleum, smelting and alloying processes and waste incineration. The natural concentration of lead in soil is 2200 $\mu g/g$. There has been large number of studies concerning the concentration of lead in natural surface waters. Concentrations of lead in natural water are generally very low (1-3 $\mu g/L$), rising up to approximately 73 mg/L in water draining from area of lead numeraligation [19].

2.2.3 Domestic environment

The domestic environment, in which infants and children spend the greater part of their time, is of particular importance as a source of lead intake. Dust in homes could contain lead from lead painted surfaces that rub against each other. Windows and doors are a large source of this type of lead dust. This dust has been danger to children who play in the dust and put things in their mouth. Other lead exposure can result from sources such as toys, furniture, and older linoleum. It may be found in tableware as diverse as: ceramic dishes, bean pots, crystal, pewter, brass and enamel metal-ware, certain types of glazed and pewter dinnerware, and lead paint decorated drinking glasses. In addition to the exposure from general environmental sources, some infants and young children, as a result of normal, typical behavior, can receive high doses of lead through swallowing of non-food items. Pica, the habitual ingestion of non-food substances, which occurs among many young children, has frequently been implicated in the etiology of lead toxicity.

2.2.4 Soil and dust in and about the home

The extent of the contribution of inhaled airborne-lead to the lead burden of children is probably small. However, lead-containing particles that deposit from the air can be responsible for high concentrations of lead in dust that children ingest [20]. A study of urban and suburban infants (USA) followed from birth to 2 years of age found that the average blood lead levels highly correlated with amounts of lead in indoor dust, top soil, and paint in their immediate environment. Children living near high-level sources of lead such as smelters are at high risk from lead poisoning [21]. Exhaust from vehicles using leaded gasoline is a common source of atmospheric lead which contributes to the lead content of dust. Data from the United States Second National Health and Nutrition Examination Survey (NHANES II) indicate that leaded gasoline was a more significant source of lead than previously thought. Rosner and Markowitz [22], using data from this study, correlated major reductions in the amounts of lead added to gasoline sold in the United States with significant reductions in children's blood lead levels. A similar relationship between leaded gasoline sales and umbilical cord blood lead levels was shown by Rabinowitz and Needleman [23]. However, other studies have indicated that the influence of lead from gasoline on blood lead levels may be relatively low. In general, lead in soil and dust appears to be responsible for blood lead levels in children increasing above background levels when the concentration in the soil or dust exceeds 500-1,000 ppm [21].

Lead-based paint in the home has been and continues to be the major source of high-dose lead exposure and symptomatic lead poisoning for children, in spite of the fact that the use of lead in interior paints has been restricted in some countries for many years [24]. In the past, some interior paints contained 20-30% lead and these paints remain in many older homes. Overt lead poisoning,

when it occurs, is usually seen in children under 6 years of age who live in deteriorated older housing [25, 26].

2.2.5 Industries

Lead inhalation and ingestion are particular hazards for workers in lead industries including mining, smelting, battery breaking, petrol refining. Metal repair or foundry work, chemical manufacture, ceramics and jewelry work. Industries which use lead or lead-based products also present hazards for workers. These include radiator and automotive repair, plumbing and construction.

2.3. Intake

Children are more vulnerable to exposure to lead than adults because of metabolic and behavioral differences [27]. The degree to which individual sources of lead contribute to the intake of lead by infants and children varies according to its availability in particular environmental circumstances. While lead in air, food, and water generally is at lower levels than lead in paint, soil, and dust, the former contribute to the background or baseline level which determines how much extra lead is needed from other sources before toxicity ensues. In most areas of the world, the intake of lead through drinking water is low, less than 0.05 μmol/day, but intake may be higher in geographic regions with combination of "soft" or acidous water and lead containing plumbing system. It has been estimated that in the United States, an average two years old child may receive 44% of his daily lead intake from dust, 40% from food, 14.6% from water and beverages, and 1% from inhaled air [28].

2.3.1 Food

The higher food intake relative to size and the higher metabolic levels and greater motor activity compared to adults, leads to higher dietary lead consumption. Reported intakes of lead from food are quite variable. However, developing a reasonable estimate of lead in the diet is a continuing problem because of (1) methodological weaknesses in the accurate analysis for lead in food and (2) the need for good dietary survey data.

Intake of lead from food varies within 0.5 to 0.15 μmol/day. In general canned foods may contain more than twice the lead contents of uncanned food.

2.3.2 Air

Inhaled lead contributes little to the background body burden compared to intake from food, water, beverages, and dust. Airborne lead, however, represents an important source of lead exposure when deposited in dust and dirt [29]. However, different studies reach widely different assessments of the contribution of air lead to food lead and hence body burden [29].

2.3.3 Dust

Dust contributes a greater proportion of lead to the background body burden of young children than to adults and older children because of their greater tendency for ingesting dust due to their greater hand-to-mouth activity. It has been calculated that dust contributes only 7 to 11% of the baseline lead in adults, but 44% in 2 years old children [30-32].

2.3.4 Intake of lead shot

Lead toxicity from gunshot wound is a rare complication. Gunshot wounds in adults and children may lead to signs and symptom of lead toxicity. It occurs when body fluids, especially synovial cavity fluids, dissolve lead from the bullets, resulting in absorption and toxicity. Metabolic stress, infection, or alcoholism can also enhance absorption Lead shot taken by birds into their gizzards is a source of severe lead contamination. It results in high organ levels of lead in blood, kidney, liver, and bone.

2.4. Accumulation

Lead in the environment is strongly adsorbed onto sediment and soil particles reducing its availability to organisms. Because of the low solubility of most of its salts, lead tends to precipitate out of complex solutions. The term bioaccumulation indicates that organisms take up chemicals to a greater concentration than that found in their environment or their food. Bio-concentration factor is a quantitative way of expressing bioaccumulation: the ratio of the concentration of the chemical in the organism to the concentration of the chemical in the environment or food. Bio-magnification refers, in this document, to the progressive accumulation of chemicals along a food chain.

In aquatic and aquatic/terrestrial model ecosystems, uptake by primary producers and consumers seems to be determined by the bioavailability of the lead. Bioavailability is generally much lower whenever organic material, sediment, or mineral particles (e.g., clay) are present. In many organisms, it is unclear whether lead is adsorbed onto the organism or actually taken up. Consumers take up lead from their contaminated food, often too high concentrations, but without bio-magnification [33].

Sources and various routes by which an individual can get exposed to lead have been summarized in Figure 1.

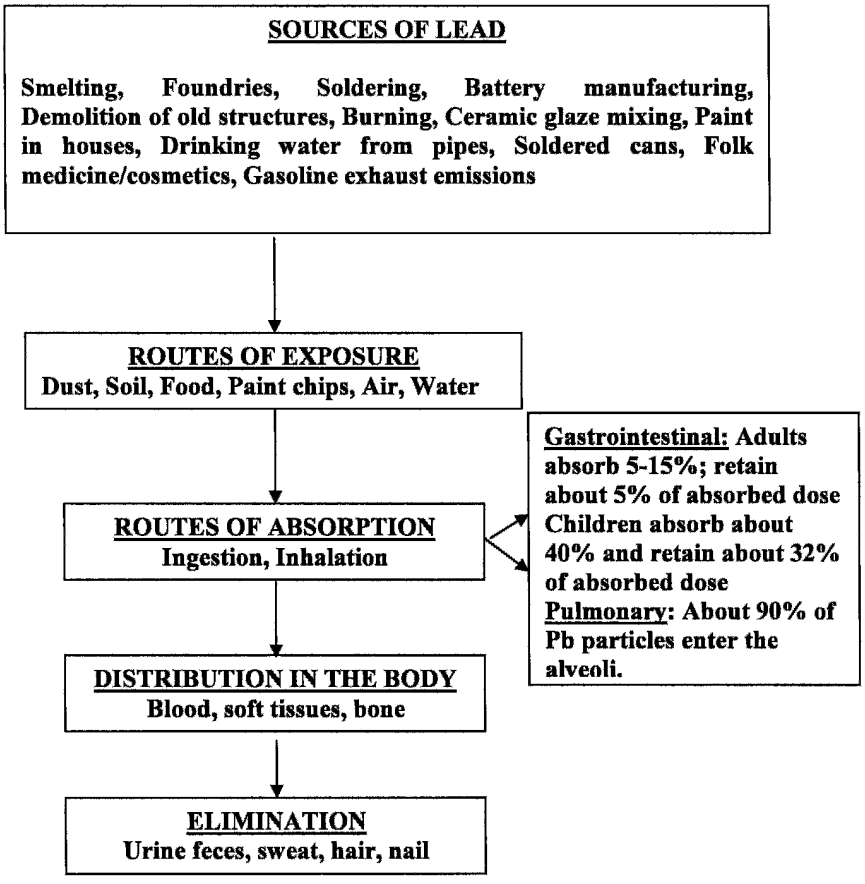


Figure 1. Sources and route of lead exposure

2.4.1 Accumulation by aquatic organisms

The uptake and accumulation of lead by aquatic organisms from water and sediment are influenced by various environmental factors such as temperature, salinity, and pH, as well as humic and alginic acid content. In contaminated aquatic systems, almost all of the lead is tightly bound to sediment [34]. Only a minor fraction is dissolved in the water, even interstitial water between the sediment particles. The lead uptake by fish reaches equilibrium only after a number of weeks of exposure. Lead is accumulated mostly in gill, liver, kidney, and bone. Fish eggs show increasing lead levels with increased exposure or concentration, and there are indications that lead is present on the egg surface but not accumulated in the embryo.

In contrast to inorganic lead compounds, tetra alkyl lead is rapidly taken up by fish and rapidly eliminated after the end of the exposure.

2.4.2 Accumulation by terrestrial flora and fauna

In bacteria, the majority of lead is associated with the cell wall. A similar phenomenon is also noted in higher plants. Some lead that passes into the plant root cell can be combined with new cell wall material and subsequently removed from the cytoplasm to the cell wall. Of the lead remaining in the root cell, there is evidence of very little translocation to other parts of the plant because the concentration of lead in shoot and leaf tissue is usually much lower than in root. Foliar uptake of lead occurs, but only to a very limited extent. Deng et al. studied the lead tolerance in six species of wetland plants and found that this special feature in these plants could be due to the special root anatomy in wetland plants, the alleviated metal toxicity by the reduced rooting conditions and the relatively high innate metal tolerance in some species [35].

In animals, there is a positive correlation between tissue and dietary lead concentrations, although tissue concentrations are almost always lower. The distribution of lead within animals is closely associated with calcium metabolism.

Lead shot is typically trapped in the gizzard of birds where it is slowly ground down resulting in the release of lead [36].

The tetravalent organic form of lead is generally more toxic than the divalent, inorganic form, and its distribution in organisms may not specifically follow calcium metabolism.

2.4.3 Accumulation in the vicinity of highways and in urban areas

Lead concentrations are highest in soils and organisms close to roads where traffic density is high. The lead measured is inorganic and derives almost exclusively from alkyl lead compounds added to petrol. The lead in the soil and in vegetation decreases exponentially with the distance from the road. Lead is also found in the sediments of streams in the vicinity of highways. Lead contamination increases lead levels in plants and animals in areas close to roads.

These levels are positively correlated with traffic volume and proximity of roads.

2.4.4 Accumulation of lead from industrial sources

Terrestrial and aquatic plants accumulate lead in industrially contaminated environments. In aquatic plant species, lead uptake can occur from both water and sediment, although uptake from sediment usually predominates. Lead levels decrease with distance from the source and are lowest during the active growing season in terrestrial plants. Mosses accumulate lead from the atmosphere and are often used as biological monitors of airborne lead. Baptista et al. reported that lichens are most suitable in bio-monitoring studies. The exposure of detached lichen allows the accurate measurement of the exposed area/volume so it can be useful to relate atmospheric deposition rates with the lichen metal content [37].

3. OCCUPATIONAL LEAD POISONING

Occupational lead poisoning has been a recognized health hazard for more than 2,000 years [38]. Characteristic features of lead toxicity, including anemia, colic, neuropathy, nephropathy, sterility and coma, were noted by Hippocrates and Nikander in ancient times, as well as Ramazzini and Hamilton in the modern era [39]. Physicians have gained an extensive understanding of the causes, the clinical presentations and the means of preventing lead poisoning. However, it remains one of the most important occupational and environmental health problems [40].

Lead serves no useful biologic function in the human body. Over the past several years, concern has increased over the health effects of low-level lead exposure and the "normal" body burden of lead. In the occupational setting, the present "no-effect" level for lead exposure is currently being re-evaluated as more sensitive measures of the physiologic effects of lead are made available through clinical investigations [41]. Based on current knowledge of the health effects of lead in adults, the U.S. Public Health Service has declared a health objective for the year 2000: the elimination of all exposures that result in blood lead concentrations greater than 25 μg per dL (1.20 μmol per L) in workers [42].

Lead and lead compounds play a significant role in modern industry, with lead being the most widely used nonferrous metal [43]. A wide variety of industrial population is at risk of occupational exposure to lead (see Figure 2). According to estimates made by the National Institute of Occupational Safety and Health (NIOSH), more than 3 million workers in the United States are potentially exposed to lead in the workplace. Occupational exposure to lead in general industry is regulated by the 1978 Occupational Safety and Health Administration (OSHA) Lead Standard. The general industry standard specifies permissible limits on airborne lead exposure, as well as blood lead levels. A construction standard, recently extended to cover workers in the construction

industry, has slight differences in detail. However, enforcement of both standards is inadequate, and significant occupational exposure remains widespread [44].

Major Occupations and Industries Associated with Lead over exposure	
❖ Battery manufacturing ❖ Chemical industry ❖ Construction workers ❖ Demolition workers ❖ Firing-range instructors ❖ Foundry workers ❖ Gas-station attendants ❖ Gasoline additives ❖ Production Jewelers ❖ Lead miners ❖ Lead smelters and refiners	❖ Pigment manufacturing ❖ Pipe fitters ❖ Plastics industry ❖ Pottery workers ❖ Printers ❖ Radiator repair ❖ Rubber industry ❖ Soldering of lead products ❖ Solid waste production ❖ Stained-glass makers ❖ Welders

Figure 2. Industries and occupations associated with possible lead exposure

Any general industry worker found to have a single blood lead level of 60μg/dL or greater, or an average blood lead level of 50μg/dL or greater must be removed from the high-exposure job (termed "medical removal protection"). The "removed" worker should subsequently receive more frequent medical evaluation and blood testing. A worker is not allowed to return to a job with the potential for high lead exposure until his or her blood lead level has fallen below 40 μg/dL (1.95 μmol/L) on two successive tests [44].

4. BIOLOGICAL ASPECTS

4.1. Lead Metabolism

4.1.1 Absorption

Lead may be absorbed into the body by ingestion [13, 45], inhalation [46], or through skin [47]. The absorption of lead from different sources is dependent on many factors, such as amount of lead presented to portals per unit time and the physical and chemical states in which lead is presented. It is also influenced by factors such as age and physiological status. For most individuals the major route of absorption is the gastrointestinal tract. Studies of ingested lead indicate

that not more than about 10% are absorbed from the gastrointestinal tract [48] and that most passes out in the faeces. Intestinal absorption of lead by children is much greater than in the adults. Nutritional iron deficiency enhances lead toxicity, thereby giving concern those pregnant women and young children may be more susceptible to lead toxicity [49, 50]. Also vitamin D stimulates lead absorption unlike inorganic lead compounds, lipid soluble compounds such as tetraethyl lead and lead naphthalenate penetrate the skin to a significant degree. Lead absorption through the skin is different from gastrointestinal lead absorption with lead transported first into the plasma and rapidly concentrated into the extra cellular fluid pool of sweat and saliva without significant uptake by erythrocytes. Gastrointestinal absorption of lead is lower in adults than in children, with an estimated 10 to 15 percent of lead in an adult's diet absorbed gastro-intestinally. The degree of lead absorption is increased considerably with fasting or in persons whose diet is deficient in calcium, iron, phosphorus or zinc [43].

4.1.2 Distribution and Retention

The model most commonly relied on for the distribution and retention of lead in man is that proposed by O'Flaherty [51], which is based on the pharmacokinetics (PBPK) model of lead uptake and disposition in children and adults. Blood contains lead in a non-diffusible form bound to erythrocytes and in a diffusible form in plasma. Plasma occupies a central position in the distribution equilibrium and would be expected to reflect the concentration of lead in all the body tissues: Lead absorbed into the body, whether by oral ingestion or by inhalation enters the blood stream where it rapidly becomes attached to red blood cells or is transported to soft tissues. Subsequently, it is transferred to bone where the reservoir is large with long half-life. In the absence of significant previous exposure, lead within red blood cells was found bound primarily to hemoglobin [52]. Lead in blood is primarily bound to RBC (99%) rather than plasma [53]. Within the cell, 50% is bound to hemoglobin [4] and other to proteins [54]. Iron deficiency is well documented to increase susceptibility to lead intoxication [43].

The concentrations of lead in human tissues and cellular organelles have been examined carefully. Some investigators have suggested a preference for binding to mitochondria. Among body organs the greatest percentage of lead is taken into the kidney followed by the liver and the other soft tissues such as heart and brain but the lead in the skeleton, represents the major body fraction [55, 56]. Within the skeleton, lead is incorporated into the mineral in place of calcium. There it accounts for about 94-95% of the total body burden in adults and about 70% in young children [57]. Lead is readily transferred across the placenta and the concentration of lead in the blood of new-born children is similar to that of their mothers, suggesting that lead come into equilibrium between the mother and foetus [58] and the increased mobilisation of bone lead

during pregnancy may also continue to increase [45, 59, 60]. Lead also crosses the blood-brain barrier and a study by Antonio et al. [61] showed that the plasma lead fraction is in equilibrium with that in the CSF. Assuming that the blood-brain barrier is functioning normally, plasma lead concentration is a good indicator of CSF lead concentration, and therefore this parameter is a reliable index of potential lead transfer to brain tissues [61]. Studies on the immune system reveal that orally administered inorganic lead affects the immune system by depressing antibody response, serum IgG and decreased thymus weight. More studies are needed to explore further details.

Number of factors are known to increase bone turnover such as pregnancy, lactation, chemotherapy, tumour infiltration of the bone or post menopausal osteoporosis may be associated with the mobilization of lead in stores, leading to chronic lead toxicity. Hypothyroidism too is known to increase bone turnover, it has rarely been implicated in the pathogenesis of lead poisoning.

4.1.3 Excretion

Inorganic lead is not metabolized and the excretion is low primarily through urinary tract. Absorbed lead is eliminated primarily via the kidney in the urine (about 76%) and to a lesser extent by the gastrointestinal tract (about 16%) through biliary secretion [40]. Other routes for elimination (hair, nails, sweat and exfoliated skin) account for approximately 8% [19]. Lead is also excreted in milk in concentrations of upto 12 μg/L. In general, lead is excreted very slowly from the body with its biological half-life estimated at 10 years, thus facilitating accumulation in the body.

5. BIOCHEMICAL AND TOXICOLOGICAL EFFECTS

5.1. Lead impairment of normal metabolic pathways

Lead is distributed in all cells. The biochemical basis for lead toxicity is its ability to bind the biologically-important molecules, thereby interfering with their function by a number of mechanisms. Enzymes may bind lead resulting in a adverse function. Lead binds to sulfhydryl and amide groups, frequent components of enzymes, altering their configuration and diminishing their activities. Lead may also compete with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium. At the sub-cellular level, the mitochondrion appears to be the main target organelle for toxic effects of lead in many tissues. Lead has been shown to selectively accumulate in the mitochondria and there is evidence that it causes structural injury to these organelles and impairs basic cellular energetic and other mitochondrial functions [62].

Lead has been reported to impair normal metabolic pathways in children at very low blood levels [63, 64]. At least three enzymes of the heme biosynthetic pathway are affected by lead and at high blood lead levels the decreased heme

synthesis which results leads to decreased synthesis of hemoglobin. Blood lead levels as low as 10 μg/dL have been shown to interfere with one of the enzymes of the heme pathway, δ-aminolevulinic acid dehydratase. No threshold for this effect has been established. Alterations in the activity of the enzymes of the heme synthetic pathway lead to accumulation of the intermediates of the pathway [65]. There is some evidence that accumulation of one of the intermediates, δ- aminolevulinic acid, exerts toxic effects on neural tissues through interference with the activity of the neurotransmitter gamma-amino-butyric acid (GABA) [66]. The reduction in heme production per se has also been reported to adversely affect nervous tissue by reducing the activity of tryptophan pyrollase, a heme-requiring enzyme. This results in greater metabolism of tryptophan via a second pathway which produces high blood and brain levels of the neurotransmitter serotonin [67]. Red cell pyrimidine-5'-nucleotidase activity in children is inhibited at blood lead concentrations of 10-15 μg/dL and no threshold was found even below these levels [68]. Lead interferes with vitamin D metabolism, since it inhibits hydroxylation of 1, 25-hydroxy-vitamin D to produce the active form of vitamin D. The effect has been reported in children at blood levels as low as 10-15 μg/dL [69].

5.2. Target organs or systems

Lead is a poison that affects virtually every system in the body. Children are more vulnerable to lead exposure than adults because of the frequency of pica, hand-to-mouth activity, and a higher rate of intestinal absorption and retention. Lead is a cumulative poison. It produces a range of effects, primarily on the hematopoietic system, the nervous system, and the kidneys.

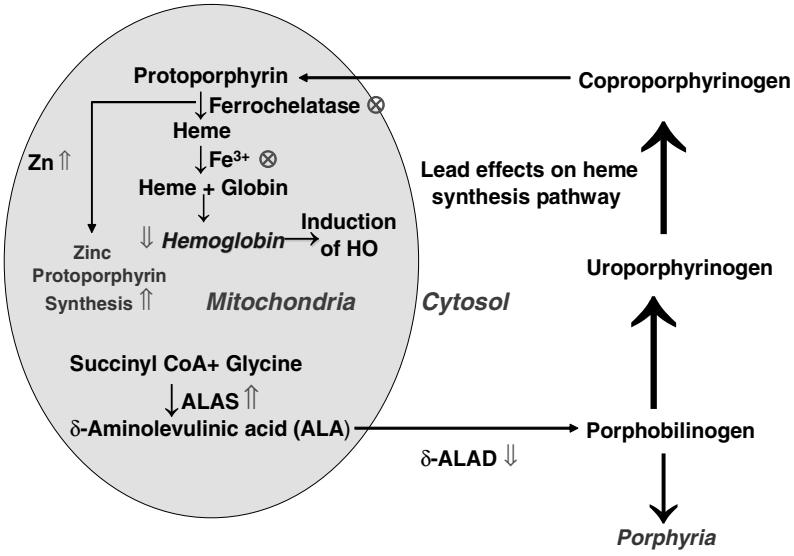
5.2.1 Hematopoietic System

The relationship between lead and heme pathway enzymes has been well studied. This pathway is found in all cells. Three of the seven enzymes in the production of heme are down regulated by lead, resulting in the dose dependent diminished production of heme and in the accumulation of precursor molecule. The hematological effects of lead can be attributed to the combined effect of (a) inhibition of hemoglobin synthesis and, (b) Shortened life spans of circulating erythrocytes. These effects may result in anaemia, which may be mild, hyper, chronic and sometimes-microcytic anaemia. Anaemia is also associated with shortened red blood cells life span. Basophilic stippling of RBC is a feature of lead induced anaemia. The adverse effects of lead appear even with blood concentration as low as 10μg/dL [69, 70].

Lead inhibits many stages in the pathway of heme synthesis (see Figure 3). δ-aminolevulinic acid dehydratase (ALAD), which, catalyses the formation of porphobilinogn from δ-aminolevulinic acid (ALA) and ferrochelatase, which incorporates iron into protoporphyrin [71]. It is suggested that the inhibition of ALAD can occur at blood lead as low as 5 μg/dL. At higher concentration

ALAD inhibition is more pronounced (90% at blood lead 55 μg/dL). ALA in urine has been used for many years as indicator of exposure, inhibition of hematopoiesis among industrial workers, and the diagnosis of lead poisoning. A significant correlation coefficient between lead in blood (and lead in urine) and ALA-U or ALA-D has been suggested [72, 73]. Ferrochelatase is the enzyme that catalyzes the incorporation of iron into the porpohyrin ring. If as a result of lead toxicity, the enzyme is inhibited and its pathway is interrupted, or if adequate iron is not available, zinc is substituted for iron, and zinc protoporphyrin concentration is increased. A dose related elevation of zinc protoporphyrin (ZPP) in lead workers has been documented [74, 75]. Many studies have reported the elevation of ZPP as being exponentially correlated with blood lead levels in children peak ZPP levels lag behind peak levels of blood lead. The critical target, however, seems to be the enzyme's heme synthesis, essential for the insertion of iron into the precursor, protoporphyrin IX. The major consequences of this effect, which have been evaluated in both adults and children, are reduction of hemoglobin and the inhibition of cytochrome P 450-dependent phase I metabolism. Lead inhibits normal hemoprotein function in both respects, which results in basophilic stippling of erythrocytes related to clustering of ribosome and microcytosis. The threshold for this effect in children is approximately 15 μg/dL [69, 76, 77]. Compared with adults, children are especially in their first year, develop certain toxic effects at lower blood lead levels and lead induced anaemia has been related to age. The mechanism for shortened erythrocyte survival is not well understood. The degree of shortening correlates with levels of anaemia, coproporphyrenuria and blood lead. Few characteristics of the erythrocyte membrane are altered during exposure to lead. Inhibition of erythrocyte membrane Na^+-K^+ATPase with increased loss of intra cellular potassium occurs in people with only moderately elevated lead exposure. Thus, Na^+-K^+ ATPase suppression may be a function of lead binding protein [78, 79].

One of the earliest observed hematological effects of lead was the occurrence of visible dense material in the erythrocytes described as "basophilic stippling". It was established that these aggregates are degradation of products of ribonucleic acid. Basophilic stippling also occurs in subjects who have generally transmitted deficiency of pyrimidine-5-nucleotidase (Py-5-N) an enzyme mediating the phosphorolysis of pyrimidine nucleotides. With the inhibition of this enzyme, nucleotides accumulate in the erythrocytes. The resulting accumulation of aggregates of incompletely degraded ribosomal materials accounts for the phenomenon of basophilic stippling [80]. It can thus be said that the effect of lead on Py-5-N activity is analogous to the effect on ALAD activity in the sense that the effect is noted at levels of exposure far below those known to have any effect on normal physiological function.



Abbreviations: ALAS- Aminolevulinic acid synthase; ALAD- Aminolevulinic acid dehydratase; HO- Heme oxygenase

Figure 3. Effects of lead on heme-synthesis pathway (Source: Kelada et al. Am. J. Epidemiol., 154, 2001.

5.2.2 Renal Effects

Lead-induced chronic renal insufficiency may result in gout. A direct effect on the kidney of long-term lead exposure is nephropathy. Impairment of proximal tubular function manifests in aminoaciduria, glycosuria, and hyperphosphaturia (a Fanconi-like syndrome). There is also evidence of an association between lead exposure and hypertension, an effect that may be mediated through renal mechanisms [81, 82]. Because of the rich blood supply in the kidney in relation to its mass, this organ in particularly liable to damage from toxic substances. Overt effects of lead on the kidney in man and experimental animals, particularly the rat and mouse, begin with acute morphological changes consisting of nuclear inclusion bodies or lead protein complexes and ultra structural changes in organelles, particularly mitochondria. Dysfunction of proximal renal tubules (Fanconi syndrome) as manifested by glycosuria, aminoaciduria and hyperphosphaturia in the presence of hypophosphataemia and rickets was first noted in acute lead poisoning [19, 83]. Long-term exposure to lead may give rise to the development of irreversible functional and morphological renal changes. This includes intense interstitial fibrosis, tubular atrophy and dilation and the development of functional as well

as ultra structural changes in renal tubular mitochondria [84, 85].

The symptoms resulting from lead poisoning are subtle, and often the patients remain asymptomatic until significant reductions of renal function have occurred. Chronic exposure to high levels of lead results in irreversible changes in the kidney, including interstitial fibrosis, tubular atrophy, glomerular sclerosis, and ultimately renal failure. These changes are non-specific and common to many other type of renal injury therefore it is essential to monitor increased lead burden before making a diagnosis of lead nephropathy. In general, individuals with blood lead levels of 60 μg/dL or more are at a definite risk of developing renal failure. Although, recently it has become evident that blood lead levels as low as 10 μg/dL, previously considered to be safe may also be associated with renal functional abnormalities. It is still controversial whether exposure to lead in childhood is associated with the occurrence of nephropathy in the later life. One interesting feature of chronic renal failure is the frequent association with gout [86]. High level of plasma urate is believed to be underlying cause of the joint disease, and generally attributed to the lower renal clearance of urate and increased urate reabsorption rather than impaired tubular re-absorption [86]. The effect of acute lead exposure has also been studied in animals principally in adult rodents, and aminoaciduria and phosphaturia have been demonstrated at very high dose levels [87].

5.2.3 Neurological and neurobehavioral Effects

The most vulnerable target of lead poisoning is the nervous system. In children, neurological deficits have been documented at exposure levels once thought to cause no harmful effects. In addition to the lack of a precise threshold, childhood lead toxicity may have permanent effects [88-90]. The effect of lead on cognitive and behavioural development of the central nervous system (CNS) is the critical effect on infants and children and is the focus for current prevention strategies [1].

- Effects in children generally occur at lower blood lead levels than in adults.
- The developing nervous system in children can be affected adversely at blood lead levels of less than 10 μg/dL.
- Neurological deficits, as well as other effects caused by lead poisoning, may be irreversible.

The generally recognized effect of lead on the CNS is encephalopathy. Headache, poor attention spam, irritability, loss of memory, and dullness are the early symptom of the effects of lead exposure on the CNS. The ability to think and reason is extremely sensitive to lead assault. The developing nervous system of the child is more sensitive to lead-induced impairment. The most serious manifestation of lead poisoning is acute encephalopathy, the symptoms of which include persistent vomiting, ataxia, seizures, pappilledema, impaired

consciousness and coma. Survivors suffered a number of neurological complications such as mental retardation, deafness, blindness and convulsions. Lead encephalopathy rarely occurs at blood lead below 100 μg/dL. A chronic form of encephalopathy has also been described [19] in which progressive mental retardation, loss of motor skills, and behavioural disorders occur rather than the more precipitous symptoms seen in acute encephalopathy [91, 92].

The primary effect of lead on the peripheral nervous system is reduced motor activity, muscular weakness, especially of the exterior muscles, tremor fatigue, and lack of muscular co-ordination. Segmental demyelination and possibly axonal degeneration follow lead induced Schwann cell degeneration. One common feature of lead neuropathy and one of the characteristic features of this disorder in adults is wrist drop due to paralysis of the distal, upper extensor muscles, which are innervated by the radial nerve [93].

Results of few longitudinal studies revealed that disturbances on early neurobehavioral development occur at levels well below those considered safe or even normal. To increase the analytical power of shidles reported to date, Needleman and Gatsonis [94] performed cross sectional epidemiological studies relating children's I.Q. to blood lead levels at low doses. Each of the studies controlled non-lead factors or covarialis. The result supported the hypothesis that lead impairs children's I.Q. at low doses. Lead has an adverse health effect on neurobehavioral and cognitive development at blood lead levels in the 10-15 μg/dL range in infants, children and the foetus, and perhaps at even lower levels. The magnitude of I.Q. decrements is dose related [95]. It is suggested that immature endothelial cells forming the capillaries of the developing brain are less resistant to the effects of lead than capillaries from mature brains. These cells permit fluid and cations including lead to reach newly formed components of the brain, particularly astrocytes and neurons [96, 97]. Neurochemical studies in experimental models have shown that lead, in the absence of morphologic changes, produces defects in neurotransmission through inhibition of cholinergic function, possibly by reduction of extra cellular calcium [98]. Other noted changes in neurotransmitter function include impairment of dopamine uptake by synaptosomes and impairment of the function of the inhibitory neurotransmitter γ- aminobutyric acid [99, 100]. The effect of lead on cholinergic system is important because of clinical evidences of lead induced peripheral neuropathy. Few studies indicate that exposure to lead at an early state produced no alterations in acetylcholine (Ach) concentrations in various brain regions. The level of its precursor, however, was shown to be reduced. Both choline and Ach remained uninfluenced in mouse forebrain, rat cerebellum, hippocampus, mid-brain, post-medulla, cortex and striatum. The high affinity transport of choline has been shown to be inhibited by lead using synaptosomes prepared for mouse forebrain, low affinity transport being unaffected. The higher brain lead level may be associated with a reduced effect on cholinergic parameters with respect to steady state of Ach levels, cholinesterase and release mechanisms.

There are also some experimental evidences to suggest that the fetal brain may have greater sensitivity to lead than the mature brain [101-103]; Rat pups readily develop encephalopathy during the first few days of life, and resistance to lead induced neurotoxicity occurs after 18-20 days of age. The development of resistance to lead encephalopathy is related to the formation of lead-protein complexes (inclusion bodies) in astrocytes, and sequesters lead away from mitochondria [104]. Thus role of lead protein complexing is similar to that postulated in renal tubular epithelial cells as mechanism for permitting the kidney to excrete lead without cellular toxicity [105]. The lead protein complexes are thought to contain a number of richly acidic proteins [106, 107].

Measure of motor activity has been shown to very sensitive to the effects of lead [94] and thus locomotor activity may be used to assess lead-induced behaviour toxicity. Studies have confirmed that inorganic lead is distributed among other nuclei, in the striatum and cerebral cortex [108, 109]. Autoradiographic studies have shown a diminished evoked release of dopamine in synaptosomal isolated from the striatum of animals exposed to lead [110]. Correa et al. [111] recently suggested that lead produces several distinct effects on CNS and the various effects of lead may follow diverse time courses. The effect of lead on cognitive and behavioural developments is the critical effect on infants and children and is the focus for current prevention and intervention strategies. An increased prevalence of symptoms associated with peripheral nerve and visual sensory function, neuromuscular and sensory nerve functions and other peripheral problems may affect few neuropsychological tests especially those that call for motor speed, dexterity and visuo spatial skills. The most sensitive indicators of adverse neurologic outcomes are psychomotor test or mental development indices. Locomotor activity can be used to assess lead-induced behavioural toxicity as the previous studies have supported that measures of motor activity are sensitive to the effects of lead [108] and also that brain areas such as cerebral cortex and basal ganglia are affected by this metal and are involved in motor activity. Some studies have also found that lead exposure produces vulnerability of mesolimbic dopamine systems and this can result in motor behaviour alterations [112, 113]. Autoradiographic studies have also provided evidences that lead exposure decreased the density of D2 receptors in the cerebral cortex [114] and also showed a diminished release of dopamine in synaptosomes isolated from the striatum of animals exposed to lead [110]. Adults with occupational exposure may demonstrate abnormalities in a number of measures in neurobehavioral with cumulative exposures resulting from blood lead levels <40 μg/dL [115]. In a number of cognitive tests performed by Payton et al. [116], men with higher blood lead levels performed less well than men with lower blood lead levels. They also found that men with higher blood lead levels showed a slower response for pattern memory.

One of the important mechanisms known for lead induced neurotoxicity is the disruption of calcium metabolism by lead (Figure 4). Calcium is a critical

component of numerous biochemical and metabolic functions in the brain and lead may act as a surrogate for calcium, resulting in subtle disruptions of essential functions. It is a divalent cation just like lead. Calcium is important in the release of neurotransmitters, regulation of some rate-limiting enzymes of neurotransmitter synthesis, storage of transmitters in presynaptic vesicular compartments, and regulation of hormone-sensitive cyclases. Studies have shown that lead has an inhibitory effect on the peripheral nervous system through stimulus-coupled or calcium-dependent release of acetylcholine. And this inhibitory effect of lead at the neuromuscular junction and the ganglion was similar to the effect of reducing the concentration of calcium in bathing media of neural preparations; so it is not surprising that this inhibitory effect of lead can be overcame by the addition of calcium. It was also noticed that micromolar amounts of lead require millimolar increases in external calcium to counteract this effect of lead [111]. A possible brief description for the interactions between calcium and lead is that lead decreases the uptake of calcium by voltage-dependent calcium channels. If the calcium concentrations inside presynaptic terminals were decreased, calcium-dependent release of neurotransmitters would be inhibited. Several recent investigations have suggested that Ca^{2+}/calmodulin and cyclic nucleotide mediated synaptic events influence long term changes in the nervous system and play an important role in the process of memory and learning [117]. Studies have also suggested that learning selectively affects the phosphorylation of synaptic proteins, that are crucially involved in the process of memory and learning [111]. Eventually, Ca^{2+}/calmodulin and cAMP carry out these functions via the action of protein kinases [118]. The calmodulin and cAMP dependent protein kinases regulate the phosphorylation of a number of synaptic vesicle proteins of which synapsin-I is the major one. Phosphorylation of synapsin-I and other synaptic vesicle proteins, facilitates neurotransmitter release from the nerve terminals and plays an important role in neurotransmission [119]. Therefore, any alternations in phosphorylation state proteins may play a central role in permanent changes in neurons and may affect processes of memory and learning [111]. Lead can activate calmodulin and can displace calcium. Therefore, lead can interfere with calmodulin present on the surface of synaptic vesicles affecting protein phosphorylation and hence neurobehavior.

Although the understanding of the neurotoxicity of lead has advanced considerably over the past several years, there are still important research issues such as reversibility of neurotoxicity of lead exposure etc. need additional attention and constitute important future research directions.

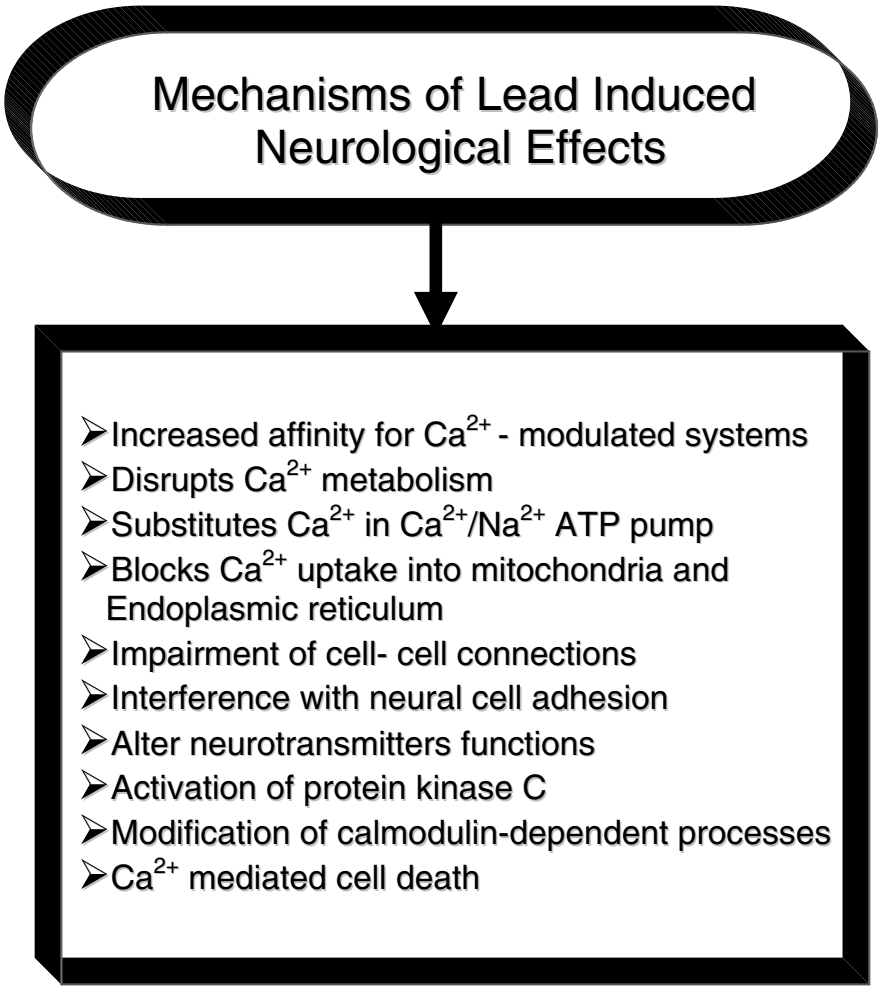


Figure 4. Possible mechanism of lead induced neurological effects.

5.2.4 Immunological Effects

Lead affects humoral immune response, functional impairments of lymphocytes and production of cytokines [120-122]. It has been well established that lead also affects humoral and cell mediated immunity, and diminishes host resistance [123, 124]. The data is limited to a few studies of immune function in lead workers. Mishra et al. [125] studied lymphocyte proliferation, natural killer (NK) cells cytotoxicity and interferon-γ production with peripheral blood mononuclear cells (PBMCs) of individual occupationally exposed to lead. They

reported inhibited lymphocyte proliferation to phytohaemagglutinin (PHA) while, no correlation between inhibition of lymphocyte proliferation and blood lead level was found in lead exposed individual. NK cell cytotoxicity remains unaffected in lead exposed workers. However, interferon-γ (IFN-γ) was significantly elevated in T cell mitogen, PHA, stimulated PBMCs culture supernatant of lead exposed individuals.

Alomran and Shleanmoon [126] have shown that lymphocytes from lead exposed workers are less responsive to PHA compared to respective controls. Kumber et al. [127] examined immune function in lead workers exposed occupationally for 4 to 30 years and controls. There were no differences between the workers and controls in serum concentration of IgG, IgA, or IgM and no correlation between blood lead levels and serum immunoglobulin levels. In addition response to the nitrogen phytohaemagglutinin (an index of T – lymphocyte function) and natural killer cells activity were not altered in workers compared with controls [128]. Large number of studies report effects of orally administered inorganic lead on components of the immune system in animals has been reviewed by the EPA [5] and in these studies marked depression of antibody response to sheep red blood cells, decreased serum IgG, impaired delayed hypersensitivity reactions and decreased thymus weights as compared with controls have been observed [5].

5.2.5 Reproductive Toxicity

Lead exerts its toxic effect directly on the developing foetus after gestation begins, and indirectly on paternal or maternal physiology before and during the reproduction process [129, 130]. Several studies have been conducted on the association between lead exposure and sterility, abortion, still births, and neonatal deaths [131, 132] but little evidence is available as to whether sub-toxic levels of lead affect fertility or cause fetal injuries in human. Several studies suggest that lead might increase the incidence of early membrane rupture and premature delivery and malformed berths [133]. Increase in blood lead reduces semen volume, semen density, and counts of total, motile and viable sperm, percentage of progressively motile sperm, levels of zinc, acid phosphatase, and citric acid and increase the percentage of pathological sperm [134, 135].These data suggest that male reproductive effects may also occur at PbB levels at the 30-40 μg/dL resulted in sub-clinical suppression of circulating cutinizing and follicle-stimulating hormone and estradiol without overt effects on general health and menstruation [133].

Abortions, miscarriages, and still births have also been reported among women working in lead industries. Pre-natal exposure to lead has been associated with toxic effects on the fetus. These include reduced gestational age, birth weight, and adversely delayed cognitive development. Significantly, sources of maternal exposure to lead may be current or the result of mobilization from bone stores remaining from previous exposures. Recent studies have

suggested that a significant amount of bone lead is mobilized, enters the circulation during pregnancy and lactation, and crosses the placenta.

5.2.6 Effects on Bone

Lead in bone is of interest for two reasons. Bone is the largest depository of the body burden of lead, and secondly, it is now recognized that lead may in fact, have an effect on bone metabolism [136]. Current concern is that lead in bone may be mobilised during a number of physiological and pathological conditions such as age, endocrine status, osteoporosis, renal disease and, in particular during pregnancy and lactation [21]. Rabinowitz [137] has suggested that the dose/ rate of lead exposure influence location and concentration of lead indifferent sites in bone, which may influence its availability for mobilisation. Other factors are maternal age, gestation age, parity and race. The major determinant for each of these factors is nutritional status, particularly dietary calcium. Pounds and co-workers [138] have reviewed the possible mechanisms where by lead may directly or indirectly alter several aspects of bone cell function [139].

For practical reasons, lead exposure in infants and children is based primarily upon measurements of lead in the blood, sometimes supplemented by measurement of lead in urine, particularly after treatment with chelating agents. These measurements correlate imperfectly with lead levels in the tissue or organ where the toxic effect may be observed. Furthermore, blood lead levels reflect only recent exposure to lead, not long-term exposure. Other methods to determine total body burden involve measurement of lead in hair, bone, or teeth.

The use of the lead content of teeth as an index of lead exposure in the general population has been considered an important advance, particularly in the investigations of the neuropsychological effects of ordinary levels of lead in the environment. This reflects lead exposure over the child's lifetime, not merely recent exposure. However, considerable variations may occur in tooth lead concentrations in different teeth from the same child, especially when teeth are different types or from upper and lower jaws. Also, there is a marked variation of lead concentration throughout the tooth. Because of these variations, there is always a need to make suitable adjustments when using teeth for assessing lead body burden.

The hematopoietic system is considered by many to be the most reliable and sensitive indicator of lead toxicity. The clinical endpoint is anemia, which apparently occurs at lower blood lead levels in children than in adults. The elevation of erythrocyte protoporphyrin (EP) has been well studied and can be reliably measured [1]. Among the biologic markers of lead toxicity, this method has been the most useful in screening programs because its measurement is not susceptible to error from lead contamination and the test can be performed on capillary blood. However, correlation with blood lead at levels below 30 μg/dL is poor, and there are a rather high proportion of false negative results. High EP

values in the absence of elevated blood lead levels may indicate iron deficiency.

Possible signs and symptoms of lead exposure and changes in major organs at different concentration of blood lead in children and adults are summarized in Figure 5 and 6.

5.2.7 Carcinogenic effects

There are number of experimental evidences where exposure to lead has been shown to cause renal tumors. Incidences of renal adenocarcinoma have been reported in lead exposed animals depending upon length and severity of exposure [140]. It is believed that males are more susceptible than females to lead induced carcinomas. Not many epidemiological studies and no conclusive evidences however, are available in the literature for the possible association between lead exposure and increased incidence of cancer. One of the possible mechanisms suggested for such effects could be related to transformation of disordered renal epithelial cell growth to renal cyst. Cells lining cysts become transformed and proliferate abnormally presumably in response to increased intracystic fluid volume. It can thus be suggested that following lead induced chronic renal failure contributes to an increase in renal adenocarcinomas.

5.2.8 Cardiovascular disorders

Lead poisoning is associated with a number of morphological and biochemical changes in the cardiovascular system. These damaging effects include an association with cardiovascular disease and chronic lead exposure. It is well established that death from cerebrovascular disease has increased among lead exposed workers. A positive association between hypertension and blood lead has also been shown in the past. A number of factors seem to indicate that the association is causal but doubts still exists on this point particular since epidemiological has had major shortcomings, (i) these studies are mainly cross-sectional and very few prospective studies available in the literature and (ii) these studies do not provide information on a number of relevant confounders [141]. Kidney disease, a risk factor for the development of hypertension is known to occur in higher incidence among lead exposed individuals.

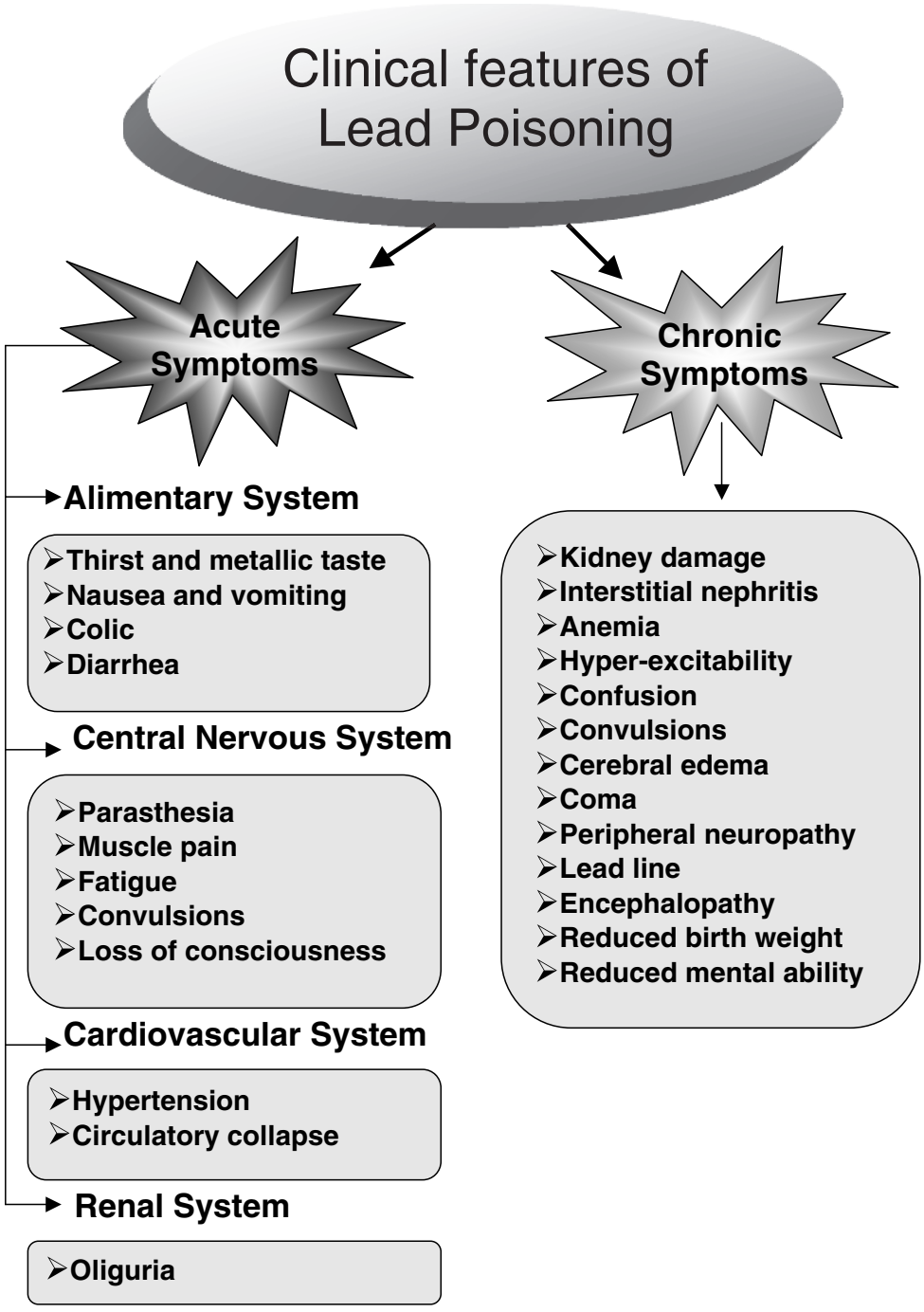


Figure 5. Signs and symptoms during lead exposure.

TOXIC EFFECTS OF LEAD IN ADULTS	
TOXIC EFFECTS	**Blood Pb level (in µg/dL)**
Nervous system: Overt clinical encephalopathy	100-120
Kidney: Atrophy and interstitial nephritis	40-100
Gastrointestinal: colic	40-60
Formation of blood cells: anemia	50
Nervous system: Learning/ IQ disruption, sensory system deficits	40
Heart and blood vessels: hypertension	<7
Biochemical: enzymes changes	3- 30
TOXIC EFFECTS OF LEAD IN CHILDREN	
TOXIC EFFECTS	**Blood Pb level (in µg/dL)**
Kidney: Atrophy and interstitial nephritis	80-120
Nervous system: Overt clinical encephalopathy	80-100
Gastrointestinal: colic	60-100
Formation of blood cells: anemia	20-40
Biochemical changes: enzymes level altered	<10
Nervous system: Learning/IQ disruption, sensory system deficits	<10

Figure 6. Toxic effects of lead in different organs in children and adults.

The pathophysiological mechanisms by which lead could induce vascular diseases are not clear. However, it could be attributed to (i) inhibition of cytochrome P-450 leading to the accumulation of lipids in vessel wall (ii) inhibition of superoxide dismutase (SOD) resulting in the elevation of serum of lipid peroxides levels which is a serious risk factor for heart diseases. Lipid peroxides are also known to promote the adherence and aggregation of platelets in the vessels of the central nervous system thereby initiating blood clotting and (iii) lead induced carcinogenicity. Formation of an atherosclerotic plaques starts from single mutated cell which is caused to stimulate proliferate by a stimulant. In the present case, lead could act as stimulants accelerating the development of atherosclerotic plaques. Goyer and Clarkson [142] also attributed lead induced cardiovascular effects to the changes in the plasma renin and in the urinary kallikrein, alterations in the calcium activated functions of vascular smooth muscles cells, including contractivity by increasing Na+-K+-ATPase activity and stimulation of Na+/Ca+ exchange pump and changes in responsiveness to catecholamines [143, 144].

5.3. Mechanism of lead induced toxicity

5.3.1 Oxidative stress

Accumulating evidences have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and weakening the antioxidant defence system of cells [145-147] (see Figure 7). Depletion of cells' major sulfhydryl reserves seems to be an important indirect mechanism for oxidative stress that is induced by redox-inactive metals [148, 149]. When GSH is reduced by lead, GSH synthesizing systems start making more GSH from cysteine via the γ-glutamyl cycle. GSH is usually not effectively supplied, however, if GSH depletion continues because of chronic exposure [150]. Several enzymes in antioxidant defense system may protect this imbalance but they also get inactive due to direct binding of lead to the enzymes' active sites, if the sites contain sulfhydryl group e.g. ALAD. Further, zinc which usually serves as a cofactor of many enzymes could be replaced by lead, thereby making the enzyme inactive.

The increased lipid peroxidation and inhibition of enzymes responsible to prevent such oxidative damage have demonstrated lead induced oxidative injury [151]. Lead induced disruption of the prooxidant/antioxidant balance could induce injury via oxidative damage to critical biomolecules. The possible mechanisms resulting in the formation of free radicals include generation of superoxide ion [152]. A significant decrease in the activity of tissue superoxide dismutase (SOD), a free radical scavenger and metalloenzyme (zinc/copper) on lead exposure have been reported [153, 154]. This could be due to an increase in lead concentration in these tissues and their possible reaction with this enzyme [155] thereby, reducing the disposal of superoxide radicals. Catalase activity too has been shown to increase in kidney.

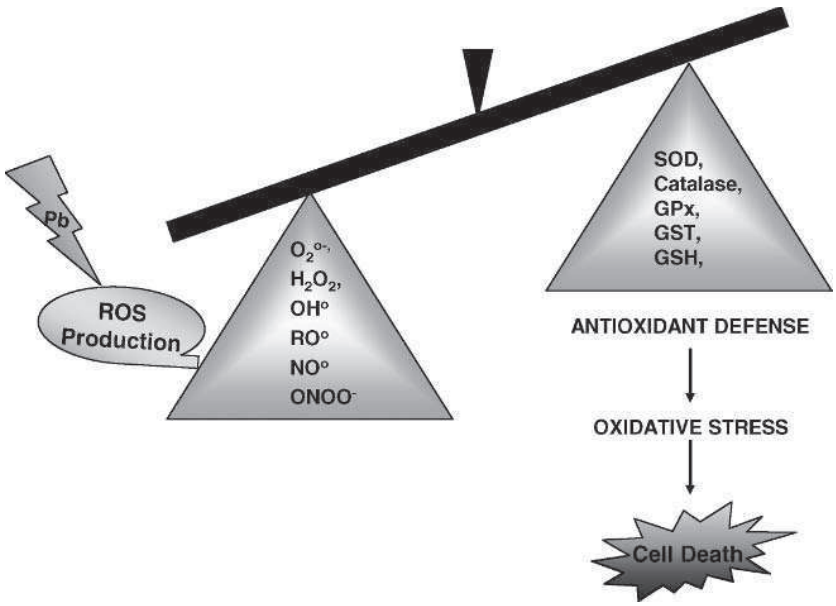


Figure 7. Lead induced oxidative stress and cell death.

Catalase is an efficient decomposer of H_2O_2 and known to be susceptible to lead toxicity [156]. Lead induced decrease in brain GPx activity may arise as a consequence of impaired functional groups such as GSH and NADPH or selenium mediated detoxification of toxic metals [157]. While, antioxidant enzyme glutathione S-transferase (GST) is known to provide protection against oxidative stress and the inhibition of this enzyme on lead exposure might be due to the depletion in the status of tissue thiol moiety. These enzymes are important for maintaining critical balance in the glutathione redox state. Malondialdehyde (MDA) levels were strongly correlated with lead concentration in the tissues of lead exposed rats [158]. The concentration of TBARS, which is a reflection of endogenous lipid oxidation level, gets increased on lead exposure. Numerous studies in the past have suggested that lead causes hemolysis and lipid peroxidation [159]. Blood levels of malondialdehyde, a product of lipid oxidation were strongly correlated with blood lead concentration higher than 35 μg/dL [160]. In pregnant women, an inverse relationship was observed between blood lead concentration and serum level of α-tocopherol and ascorbic acid [161]. The interaction of lead with oxyhemoglobin (oxyHb) has been suggested as an important source of superoxide radical formation in RBCs [162]. This may perhaps be the reason for lead induced substantial increase in the autoxidation of hemoglobin in an *in vitro* liposome model. Ercal et al. [149] postulated that antioxidant enzymes inhibited hemoglobin auto-oxidation by lead, suggesting

O_2^{o-} and H_2O_2 were involved in this process. It can be concluded that lead induced oxidative stress might be due to the interaction of lead with oxyhemoglobin leading to peroxidative hemolysis in RBC membranes. δ-aminolevulinic acid dehydratase (ALAD) is a sulfhydryl-containing enzyme that catalyzes the asymmetric condensation of two δ-aminolevulinic acid (ALA) molecules yielding porphobilinogen, a heme precursor [163]. Consequently, ALAD inhibition can impair heme biosynthesis [163] and can result in the accumulation of ALA, which may disturb the aerobic metabolism and also have some pro-oxidant activity. It has also been suggested that accumulation of ALA, resulting from inhibited ALAD activity, may undergo metal catalyzed auto-oxidization, resulting in the conversion of oxyhemoglobin to methemoglobin in a process that appears to involve the formation of reactive oxygen species such as superoxide and hydroperoxides [164]. Besides oxyhemoglobin, methemoglobin and other ferric and ferrous complexes were also shown to trigger ALA oxidation [165]. This could further be proved by the parallel reductions of ferricytochrome c and also by electron spin resonance spin trapping experiment [146, 164]. Monteiro et al. [165] also confirmed that ALA/oxyHb coupled oxidation results in ROS generation. ALA undergoes enolizaton and autoxidation at pH 7.0-8.0. The conversion of ALA keto form into the ALA enol form is shown necessary for auto-oxidation reaction because levulinic acid, without the amino group that is thought to facilitate the enolization, has not been found to be active in oxidation reaction [150, 165-167]. Decrease in blood and tissue ALAD activity and GSH concentration following lead exposure supports these hypotheses. ALAS is an enzyme which catalyses the condensation reaction of glycine plus succinyl CoA to ALA leading to increased ALA production and urinary excretion [168]. It is well known that the ALAS activity, the rate limiting enzyme of the heme biosynthesis is readily responsive to a variety of factors including lead. We reported significant inhibition of ALAS in liver which could be in part result of the inhibition of protein synthesis by lead [154].

Production of GSH is considered to be the first line of defense against oxidative injury and free radical generation where GSH functions as a scavenger and a co-factor in metabolic detoxification [169]. GSH has carboxylic groups, an amino group, a sulfhydryl group and two peptide linkages as sites for the reaction of lead (Figure 8). Its functional group, -SH plays an important role in lead binding. Several reports have demonstrated that GSH is decreased in the brain, liver and eye lens of rats exposed to lead [149].

It can thus be concluded that inhibitory effect of lead on antioxidant enzymes and glutathione appear to impair the cells' antioxidant defenses and render them more susceptible to oxidative attacks.

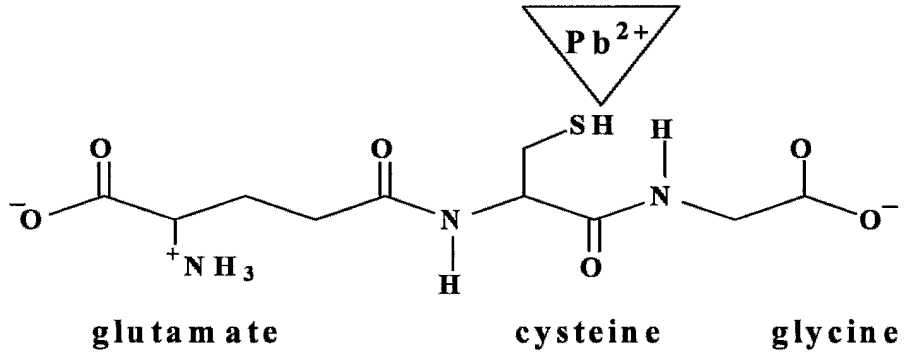


Figure 8. Lead binding at –SH group of GSH.

5.3.2 Ionic mechanisms for lead toxicity

Interaction with essential elements has been assigned to be one of the possible mechanisms of lead induced toxicity. Involvement of lead interaction with divalent and monovalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Na^{+} in the context of neurochemical effects have been shown [170]. Following section deals with brief description on interaction of lead with important monovalent and divalent cations depicting its ionic mechanisms.

5.3.2.1 Calcium

Although all of lead's toxic effects cannot be tied together by a single unifying mechanism, lead's ability to substitute for calcium [171] is a factor common to many of its toxic actions. For example, lead's ability to pass through the blood–brain barrier (BBB) is due in large part to its ability to substitute for calcium ions (Ca^{2+}). Calcium is a divalent cation just like lead. It is important in the release of neurotransmitters, regulation of some rate-limiting enzymes of neurotransmitter synthesis, storage of transmitters in presynaptic vesicular compartments, and regulation of hormone-sensitive cyclases. Experiments with metabolic inhibitors suggest that back-transport of lead via the Ca-ATPase pump plays an important role in this process [172]. More direct evidence for the role of the Ca-ATPase pump in the transport of lead into the brain has been provided by *in vitro* studies of brain capillary endothelial cells, the primary constituent of the BBB [173, 174]. Studies have shown that lead has an inhibitory effect on the peripheral nervous system through stimulus-coupled or calcium-dependent release of acetylcholine [175]. This inhibitory effect of lead at the neuromuscular junction and the ganglion was similar to the effect of reducing the concentration of calcium in bathing media of neural preparations; so it is not

surprising that this inhibitory effect of lead can be overcame by the addition of calcium.

Lead has diverse and complex action on calcium messenger system, emphasizing the importance of this pathway as a key molecular and cellular target of lead toxicity. Lead substitutes for calcium in affecting the activity of second messengers. Calmodulin, activated by calcium, stimulates several protein kinases, cyclic AMP and phosphodiesterase, and affects potassium channels [91]. Lead at nanomolar concentrations substitutes for calcium in activating calmodulin and at higher concentrations appears to reduce activity [175]. Lead's activating effects on calmodulin perturb intracellular calcium homeostasis [98], an effect with potential disruptive influences on the multiplicity of calcium-mediated processes intrinsic to normal cellular activity.

The hypotheses that lead acts competitively, but reversibly, with calcium are substantiated by these studies. It was also noticed that micro molar amounts of lead require milli molar increases in external calcium to counteract this effect of lead. A possible brief description for the interactions between calcium and lead is that lead decreases the uptake of calcium by voltage-dependent calcium channels. If the calcium concentrations inside presynaptic terminals were decreased, calcium-dependent release of neurotransmitters would be inhibited [177].

5.3.2.2 Sodium

Lead also has its effect on sodium concentrations. The concentration gradient of sodium in axons is responsible for the depolarization currents in excitatory tissues. As a result, sodium fluxes affect other ion concentrations. Moreover, high-affinity uptake of neurotransmitters and metabolic precursors are highly sodium-stimulated. Synaptosomes prepared from rodents exposed to lead *in vivo* demonstrate inhibition of sodium-dependent high-affinity uptake of choline, dopamine, and GABA [178]. Sodium also regulates the uptake and retention of calcium by synaptosomes and lead has been shown to affect the concentration of calcium inside synaptosomes. Lead can increase the retention of calcium in synaptosomes. The release of loaded calcium from these organelles was also reduced. The interactions of lead with sodium possibly explain this decrease in efflux of calcium since the release of loaded calcium is dependent on sodium concentration. Addition of sodium can increase synaptosomal uptake and rapid release of calcium.

Inhibition of an enzyme, membrane-bound Na^+, K-ATPase, increases calcium uptake by synaptosomes because the activity of this enzyme is linked to the Na-Ca exchange process. As a result, the effect of lead on the Na, K-ATPase was studied by measuring the activity of the enzyme and using 3H-ouabain as a ligand for the enzyme-receptor complex. However, no effects were reported and

the actions of lead on Na, K-ATPase to increase influx of synaptosomal calcium are not substantiated.

5.3.3 Lead and apoptosis

Apoptosis (programmed cell death) can be induced by a variety of stimuli. Apoptosis occurs when a cell activates an internally encoded suicide program as a response to either intrinsic or extrinsic signals. One of the better characterized apoptotic cascade pathways has mitochondrial dysfunction as its initiator. Mitochondrial dysfunction initiated by the opening of the mitochondrial transition pore leads to mitochondrial depolarization, release of cytochrome C, activation of a variety of caspases and cleavage of downstream death effect or proteins, and ultimately results in apoptotic cell death. While, a variety of stimuli can trigger opening of the mitochondrial transition pore and cause apoptosis, a sustained intracellular increase in Ca^{2+} is one of the better-known triggers; accumulation of lead is another. Lead disrupts calcium homeostasis, causing a marked accumulation of calcium in lead-exposed cells [91, 171]. Lead, in nanomolar concentrations, also induces mitochondrial release of calcium [179] thus initiating apoptosis.

Lead-induced apoptosis has been particularly well studied in the retina. Exposure to low to moderate pathophysiologically relevant concentrations (10 nM to 1 μM) of lead ions (Pb^{2+}) induced apoptosis in rod and bipolar cells both in cell culture [180] and in developing and adult rats [181]. Exposure to low to moderate levels of lead during development (0–21 days), resulting in blood lead levels of 19–60 μg/dL at 21 days of age, produced selective loss of rod and bipolar cells, the dying cells exhibiting signs of apoptosis. Similar results were obtained from adult rats exposed to low to moderate lead levels for 6 weeks. In all cases, the degree of cell death was age and dose-dependent, the developing retina being particularly sensitive to lead exposure. Lead-induced retinal degeneration also appeared to be related to rod-specific effects of Pb^{2+} and Ca^{2+} on rod mitochondria, suggesting that Pb^{2+} and Ca^{2+} bind to the internal metal-binding site of the mitochondrial transition pore, subsequently open the transition pore, and initiate the cytochrome C–caspase activation cascade leading to apoptosis. These effects of lead on retinal cell apoptosis may have particular functional significance, since long-term visual system deficits occur in humans, monkeys and rats following low to moderate developmental exposure to lead (20–60 μg/dL) [182].

Lead accumulation in the mitochondria can produce damage [88], the organelles mediating cellular energy metabolism. Heme biosynthesis, a function of normal mitochondrial activity, is affected by lead, with disruptive effects on synaptic transmission in the brain. However, decreased mitochondrial functioning also can transform ordinarily benign synaptic transmission mediated by glutamate into neuron-killing excitotoxicity [183]. In addition to killing brain cells via excitotoxicity and apoptosis, lead also causes toxic effects by oxidative

stress and by either directly or indirectly-produced lipid peroxidation. Lead alters lipid metabolism, inhibits superoxide dismutase and enhances lipid peroxidation in the brains of developing rats [184-186].

Chronic administration of low doses of lead to rats, at levels similar to environmental exposure in people, affects various parameters of energy metabolism in adult brain nerve endings [187]. After acute lead exposure that approximated occupational exposure (mean blood lead level 97.2 µg/dL), oxygen consumption was increased in brain synaptosomes and levels of ATP, creatine phosphate and creatine kinase were elevated, while the activity of sodium–potassium-ATPase was decreased [187].

Toxic effects of lead and possible mechanisms of all such effects have been summarized in Figure 9 and 10.

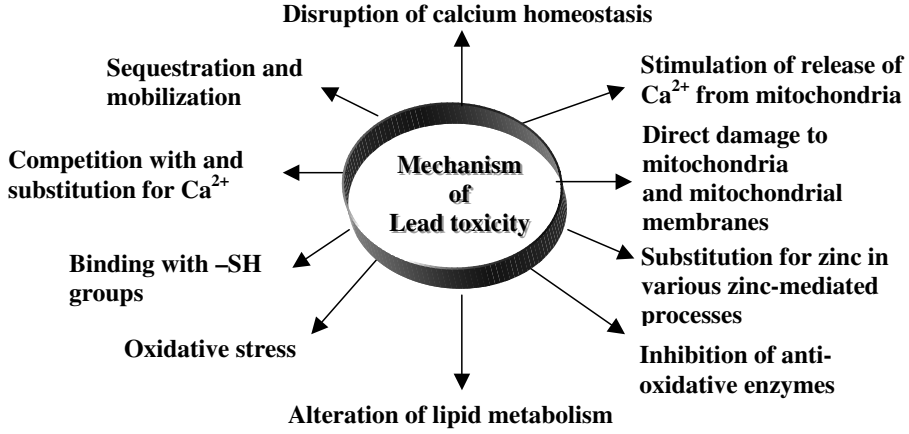


Figure 9. Mechanism of lead toxicity.

5.4 Symptoms and biochemical indicators (Diagnosis) of lead toxicity

Lead is known to cause acute, sub-chronic and chronic toxicity. The most commonly used biological marker is the concentration of lead in blood. The concentration of lead in plasma is very low and thus is not recommended.

5.4.1 Acute toxicity

Acute poisoning is uncommon. It results from inhalation of large quantities of lead due to occupational exposure among industrial workers and in children through ingestion of large oral dose from lead based paint on toys. The clinical symptoms of acute poisoning are characterised by metallic taste, abdominal pain, vomiting, diarrhoea, anaemia, oliguria, collapse and coma.

Blood lead levels: Acute lead toxicity occurs at blood levels of 100-120 µg/dL in adults and 80-100 µg/dL in children.

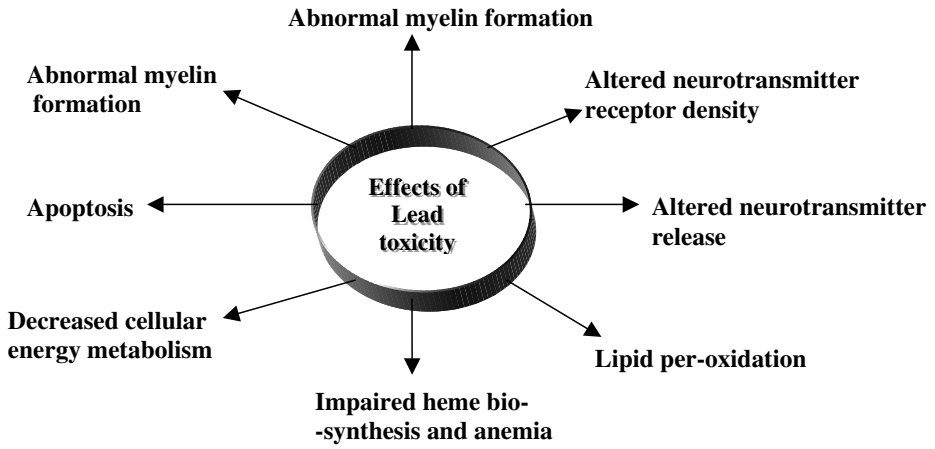


Figure 10. Effects of lead toxicity.

5.4.2. Chronic toxicity

This is more common and can be described in three stages of progression:

(i) The early stage is characterised by loss of appetite, weight loss, constipation, irritability, occasional vomiting, fatigue, weakness, gingival lining on gums and anaemia;
(ii) The second stage is marked by intermittent vomiting, irritability, nervousness, tremors and sensory disturbances in the extremities, most often accompanied by stippling of red blood cells; and
(iii) A severe stage of toxicity is characterised by persistent vomiting, encephalopathy, lethargy, delirium, convulsions and coma.

Blood lead levels: Symptoms of chronic toxicity may appear in adults at blood lead levels of 40-60 μg/dL. There are no safe levels of blood lead below which children are not affected [16]. The free erythrocyte protoporphyrin or zinc protoporphrin levels reflect impaired heme synthesis. These may therefore be increased in children with increased blood lead but they can also be increased during iron deficiency or hemolytic anaemia.

Radiological examination is not a sensitive method for diagnosis acute lead poisoning but could be used for chronic cases of poisoning. While, nerve conduction velocity should be considered when there are persistent symptoms or clinical finding suggestive of the presence of peripheral neuropathy. Neurobehavioral testing is indicated where there is persistent impairment of cognitive function and blood lead levels are usually above 80 μg/dL. Only a small minority of children with lead poisoning will have lead-related symptoms

and signs. The gastro-intestinal tract and central nervous system are the most likely areas to be involved. When blood lead concentration reaches 50 μg/dL or higher patients may complain of abdominal pain, loss of appetite, nausea, vomiting and constipation. These symptoms often persist for weeks before the diagnosis is considered. Recently it was reported that these complaints could be associated with children having blood lead levels at 20 to 45 mg/dL range as compared with lower levels and they may occur in as many as 40% of such children [188]. In contrast to adults, children with lead poisoning rarely show peripheral nervous system effects, but wrist drop and foot drops may occur particularly in patients with sickle cell disease. Central nervous system symptoms also occur. The current thinking that lead poisoning is an asymptomatic disease below a level of approximately 50 μg/dL needs to be re-examined because both gastrointestinal and behavioural disturbances occur with significant frequency at lower levels.

5.4.3 Clinical biochemical indicators

Inhibition of blood δ-aminolevulinic acid dehydratase activity (δ-ALAD), increased uptake of blood lead, and enhanced urinary excretion of lead and δ-aminolevulinic acid (δ-ALA) are the most essential diagnostic tests of lead poisoning. Other tests including blood hemoglobin, basophilic granulation of red blood cells and blood zinc protoporphyrin can also provide some essential information (see Figure 11). Lead in urine is less often used as an indicator of exposure. The urinary excretion of lead increases immediately after exposure and thus could be considered as an indicator for recent lead intake. Urinary lead excretion depends on various factors and also varies with time besides no direct conclusion about the pattern of exposure could be drawn from urinary lead excretion. 95% of the absorbed lead is incorporated in the bone and skeleton. Half time of the bone lead is more than 10 years and thus could be a useful tool for estimating long term integrated lead exposure. However, performing bone biopsies is difficult and thus this test is not generally used for large surveys. On the other hand teeth are more readily available particularly from children but also from adults and teeth lead have been used for low-level lead exposure assessment. A positive correlation too has been shown between teeth lead and blood lead level.

Radiological examination could be a tool for diagnosing acute lead poisoning as it may reveal radiographic evidence of paint chip ingestion in children but this could not be a sensitive method. Similarly nerve conduction velocity testing might also be considered when there are persistent symptoms or clinical finding suggestive of the presence of peripheral neuropathy. Neurobehavioral testing could also be useful as this may reflect changes perceptual motor speed and memory deficits which are characteristic of lead toxicity.

5.4.4 Provocative Test/$CaNa_2EDTA$ mobilisation test

High body burden of lead is demonstrated by measuring the excretion of lead in the urine after provocation with a chelating agent.

The lead mobilisation test or provocative test is considered among the most helpful tests for detecting an increased lead burden. This test consists of administration of a standard dose of calcium EDTA and collection of a 24-h urine sample for the measurement of lead content. The calcium EDTA usually is given by intravenous infusion over 1-h period in a dose of 1 g for a 70kg adult or 25 mg/kg of body weight for children [189]. Although, the mobilisation test is currently considered a sensitive method of determining the mobile and potentially toxic fraction of body lead stores and of assessing response to chelation therapy [13], its applicability for wider use in children is limited [1], since it requires hospitalization, administration of two intramuscular injections of $CaNa_2EDTA$, and 24-h quantitative urine collection, which are difficult to complete successfully in young children. In this procedure, a single injection of Ca disodium EDTA is deemed necessary, based on the presumption that total urinary lead excretion is proportional to either the body burden of lead or the amount in soft tissue. A controversy was however was raised on this test when a study performed by Cory Slechta et al. [190] suggested that a single injection of 150 mg/kg of Ca disodium EDTA may elevate brain lead content. This is an effect of special significance because the single injection protocol parallels the clinical diagnostic procedure termed the Ca disodium EDTA mobilisation test.

Figure 11. Laboratory tests to evaluate lead intoxication

- **Blood lead level**
- **Blood δ- ALAD activity**
- **Urinary ALA**
- **Erythrocyte protoporphyrin level**
- **BUN and creatinine level**

6. PREVENTIVE MEASURES FOR LEAD TOXICITY

One of the ideal strategies for "treatment" of lead toxicity is primary prevention of exposure. Treatment of lead toxicity has included chelation therapy for asymptomatic patients with certain lead values and for symptomatic patients. Simple measures to prevent lead exposure include letting tap water run two to three minutes in the morning before drawing water for use. Also, storing or serving food or beverages in lead container should be avoided. Home remodelling should be done with minimal dust production and inhalation and renovator should be aware that mechanical and or thermal methods for removing finishes create lead particulate matter that provide the greatest risk of lead exposure. Covering surfaces containing lead-free paints or other materials is the preferred method for safeguarding residents from lead exposure. Use of imported canned food or decorative serving dishes should be avoided. Such products contain higher lead concentration than do domestic products [191].

One of the best measures to minimize lead exposure is by maintaining nutritional health. Absorption of lead is increased in subjects with deficiencies in iron, zinc, vitamins (like thiamine); thus maintaining good nutrition minimizes dietary absorption of lead. For example, concomitantly iron-deficient patients must receive appropriate iron-replacement therapy.

The best non chelation therapy is the removal of subject from the source of lead exposure. In the following paragraphs, we have discussed the role of few nutritional components which have been reported to have beneficial effects in preventing lead exposure. It is however, important to evaluate the present level of lead exposure and the quantities of dietary nutrients supplements that might either augment or protect against the adverse effects of lead, so that multi-faceted components contributing to safety can be incorporated in the regulatory decision.

6.1. Role of micronutrients

Ingestion of metals like calcium, iron, zinc, copper etc. may greatly reduce the metal toxicity, while animal raised on a diet low in these metals have much higher metal concentration [192]. Since calcium deficiency potentially can result in elevated lead accumulation, maintenance of adequate dietary calcium levels appear extremely important towards minimizing susceptibility to lead toxicity? Interaction between lead and calcium occurs at several sites in the body, including cellular mechanisms that regulate ion transport across membrane. A significant increase in tissue lead, urinary delta-aminolevulinic acid (ALA) and renal intranuclear lead inclusion bodies was observed in lead exposed rats consuming low calcium [193]. Silbergeld et al. [194] found that calcium intake was negatively correlated with the blood lead level insignificantly, not much epidemiological data on the relationship between dietary calcium intake and blood lead level in normal population groups are available. Kostial et al. [195]

presented new evidence on the effect of nutritional factors on the metabolism of toxic metal in humans. They recommended adequate calcium especially for pregnant and lactating women (to prevent bone resorption) and for children (to enhance bone mass formation). The mechanism by which calcium interferes with lead absorption is not clear however; few interesting studies using ligated isolated loop technique suggest that calcium intake rather than calcium status of the animals modulates lead absorption. These studies also demonstrated that at-least in part calcium appears to inhibit lead absorption via competition for common binding sites on intestinal binding proteins.

Iron, an essential element is involved in numerous biochemical reactions. Iron functions mainly in the regulation of oxidative processes. It is a component of heme compounds that transport oxygen, cytochrome that functions in the electron transport chain and metalloprotein [196]. The influence of iron intake on lead metabolism has been studied [192]. It was suggested that subjects consuming low iron diet had tissue lead concentration significantly higher than subjects consuming adequate iron. Further, excess iron uptake decrease blood, femur and kidney lead concentration while the low iron increases the tissue lead concentration. Very limited information of whether or not neurobehavioral changes and cognitive impairment are more extreme in iron deficient, lead toxic children than in either condition are available. The role of iron and lead in heme synthesis is very well understood. Ferrochelatase activity is sensitive to both lead and iron. Piomelli et al. [197] reported that the enzyme kinetics of ferrochelatase in isolated human reticulocytes changes with both iron and lead concentration [198]. Very limited information of whether or not neurobehavioral changes and cognitive impairment are more extreme in iron deficient, lead toxic children than in either condition, are available. The role of iron and lead in heme synthesis is well-understood. The cellular basis for greater susceptibility of non-iron deficient animals to lead is that limited iron in the mitochondria apparently enhances the impairment by lead of iron utilization for heme synthesis. Additional studies have demonstrated the capacity of MT to attenuate the lead-induced inhibition of blood aminolevulinic acid dehydratase. Existence of MT-like protein in erythrocyte that binds lead and possibly protects against lead toxicity by rendering lead unavailability for retention in the target organs.

The relationship between dietary zinc and lead and its possible clinical significance are well documented. When dietary zinc is increased over requirement level, it reduces apparent metal absorption. Despite the strength of this observation, it is also possible that lead and zinc are competitive at other tissue sites, which would account for at least part of the protective effect of zinc on lead toxicity [199, 200]. The observations that zinc reversed inhibition of the lead sensitive zinc dependent enzyme δ-aminolevulinic acid dehydratase (ALAD) are in support of this alternate mechanism [201]. Flora et al. [202] investigated the influence of orally supplemented zinc in preventing or treating lead intoxication in experimental animals [202]. It was observed that oral

administration of zinc prevents appearance of lead sensitive biochemical effects and tissue absorption of lead. Border et al. [203] suggested that during concomitant industrial exposure to both zinc and lead, zinc might activate the enzyme to mask the effect of lead. Protective effect of zinc against lead toxicity could be attributed to a decrease in metal absorption in the gastrointestinal tract. Zinc has also been shown to have an antioxidant effect [204]. There are two mechanisms proposed for such an effect (i) the protection of sulfydryl groups against oxidation and prevention of reactive oxygen species (ROS) production by transition metals and (ii) further zinc supplementation could significantly compete for and effectively reduce the availability of binding sites for lead uptake. Enhanced zinc intake also increases the renal and hepatic contents of metallothionein and causes detoxification through metal binding in this form.

Selenium is an integral component of ubiquitous enzyme glutathione peroxidase, an antioxidant enzyme. This enzyme together with superoxide dismutase, catalase and vitamin E neutralised reactive oxygen species (ROS). Role of selenium lead intoxication has been rather controversial. Selenium in low dietary levels mildly protects against toxic effects of lead while, in high levels it exaggerates the lead toxicity [147]. Flora et al. [205] suggested that selenium could partly prevent or reduce the toxicity of lead during the course of simultaneous administration. Thus in brief, three possible mechanisms were proposed for the protective effect of selenium: (i) formation of an in active lead-selenide complex, (ii) stimulating radicals scavenging by increasing removal of the superoxide radical, and (iii) increasing the antioxidant capacity of cells indirectly by increasing of glutathione reductase which has a major role in maintaining a sufficient content of GSH in the reduced form [167, 206].

Copper is a component of the mitochondrial electron transport chain, functions in iron absorption and mobilization and maintenance of neurotransmitter levels in brain. Adequate intake of copper has been reported to prevent lead-induced anemia, which was developed when dietary copper was low. However, in an conflicting report animals fed low, adequate and high copper to the lead exposed rats, exhibited a pronounced decrease in lead contents in animals fed low calcium diet while, high copper diet produced an increase tissue lead accumulation. Simultaneous supplementation of copper concomitantly with lead in rats led to significant lead depletion in blood and tissues.

6.2. Role of Vitamins

Vitamins (particularly vitamin B, C and E) too are expected to play a major role in preventing the toxic effects of lead. Exposure to lead has been found to decrease vitamin contents of different tissue and blood [207]. On the other hand, supplementation of various vitamins also has been found to reduce toxic symptoms of lead. It was concluded that dietary deficiency of various vitamins B and nicotinamide enhances [208, 209], while their supplementation prevents

[210] experimental lead intoxication. Fisher et al. [211] using mammalian cell culture reported the, usefulness of vitamins in modifying the uptake, cytotoxicity and release of lead from these cultured cells. Some of the important vitamins that have been screened for their role in modulating lead and cadmium toxicity are described below.

Ascorbic acid is one vitamin, which has been studied extensively in modulating lead intoxication. Ascorbic acid (vitamin C) is known to have number of beneficial effects against lead toxicity. It acts mainly as an antioxidant molecule and its beneficial effects could be attributed to its ability to complex with lead [212], Simon and Hudges [213] reported a population-based study that indicates an inverse relation between serum ascorbic acid and blood lead levels. The authors suggest higher intake of ascorbic acid will be effective in preventing lead toxicity if a casual relationship is confirmed. It acts mainly as an antioxidant molecule and its beneficial effects could be attributed to its ability to complex with lead. Animal studies have suggested an antagonistic effect of ascorbic acid on lead absorption and toxicity and ascorbic acid may even chelate lead as effectively as EDTA [214]. However, studies in humans have shown some mixed results. In a study with 78 male workers, 38 received vitamin C and 38 were given placebo [215]. They found no effect of ascorbic acid on absorption or excretion of lead. However, 47 psychiatric patients receiving ascorbic acid and zinc showed reduced blood lead concentration [216]. Simon and Hudges investigated the association between ascorbic acid concentration and the prevalence of elevated blood lead concentration in 19, 578 participants ages 6 years and older, in National Health and Nutrition Examination Survey 1988-94 (NHANES III) [213]. The potential of ascorbic acid to counter lead intoxication may also be attributed to its ability to *in vivo* reduce the disulphides to thiol containing compounds required for the stimulation of lead inhibited heme synthetase activity

Thiamine is one of the important vitamins which have been shown to have a significant protective value against short-term lead intoxication. First report concerning the beneficial effects of thiamine was published by in early eighties by Bratton and co-authors [217] who evaluated the use of thiamine as a protective agent against short term lead intoxication in calves and observed that thiamine decreased mortality and lead accumulation in different organs of calves. However, thiamine had no therapeutic effect on inhibition of blood ALAD activity in this study. Thiamine administered subcutaneously at a dose of 100mg/calf, decreased mortality and lead accumulation in different organs of calves [217]. However, no beneficial effect on lead sensitive biochemical indices like blood ALAD was noted in this study. Further studies suggested that thiamine initially facilitated absorption and increase of lead concentration in tissues and that thiamine may also promote a rapid release of lead from tissues [218]. Beneficial effect of dietary thiamine supplement on the tissue accumulation of lead, urinary excretion of ALA and inhibition of blood ALAD

activity compared to rats fed normal thiamine or thiamine deficient diet was demonstrated by us [219]. Kim et al. [220] reported that the lead retention increased with increase in thiamine concentration. These studies further suggested that thiamine may also promote a rapid release of lead from tissue. Few other studies reported that thiamine deficiency in brain might be one of the contributing factors for the increased sensitivity to seizure in lead exposed animals [221]. A protective mechanism, such as thiamine-lead complex formation was proposed. It was suggested that thiamine might facilitate the removal of lead from body fluids and other tissues by the formation of readily excretable complexes [101, 222-224]. Despite these encouraging reports, there are still no clinical reports available on the effect of dietary thiamine supplementation on lead toxicity. Further, the mechanism of thiamine-lead interaction in the body remains unclear.

Vitamin E is an important antioxidant, which is suggested to play a physicochemical role in the stabilisation of bio-membrane by virtue of lipid-lipid interaction between the vitamin and the unsaturated fatty acids [147]. Lead poisoning has been shown to cause a marked anaemia in vitamin E deficient rats indicating a possible involvement of this vitamin in the synthesis of heme protein. Dhawan et al. [225] reported that vitamin E supplementation could prevent inhibition of blood ALAD activity and elevation of urinary ALA excretion and was effective in reducing the lead induced altered biogenic amines levels in brain during the concomitant exposure to lead. Vitamin E supplementation during concomitant lead exposure also prevented lead deposition in liver and blood. It was assumed that vitamin E may have a stimulatory effect on the enzymes ALAS which catalyses the condensation reaction of glycine succinyl CoA to ALA leading to increased ALA production and urinary excretion.It also appears that the protective effect of vitamin E in lead toxicity is attributed mainly to its antioxidant property [226]. Vitamin E, which is a low molecular mass antioxidant, interacts directly with the oxidizing radicals and protects the cells from reactive oxygen species [227, 228]. Intramuscular administration of vitamin E prevented inhibition of blood ALAD activity, elevation of urinary ALA excretion and was effective in reducing the lead induced altered biogenic amines levels in brain during the concomitant exposure lead [225]. Vitamin E supplementation during concomitant lead exposure also prevented lead deposition in liver and blood. Some of the protective effects of vitamin E also emerge directly from its antioxidant property and some through its influence on the drug metabolising enzyme system [167, 229]. Vitamin E on the other hand, suggested playing a physicochemical role in the stabilization of bio-membrane by virtue of lipid-lipid interaction between vitamins and the unsaturated fatty acid.

6.3. Role of antioxidants

Oxidative stress associated with the presence of lead in mammalian tissues and organs (predominantly blood, liver, kidneys and brain) appears to be one of the possible molecular mechanisms for lead toxicity that has been postulated by researcher. Lead induced disruption of the pro-oxidant/antioxidant balance in lead burdened tissue contributes to tissue injury via oxidative damage to critical biochemical like lipids, proteins and DNA [230]. Significant accumulation of malondialdehyde, a by-product of lipid peroxidation and depletion of GSH has been observed in both lead exposed *in vitro* and *in vivo* systems. Several studies have been reported showing how lead induced accumulation of ALA induces reactive oxygen species (ROS) generation [172, 231]. Recently, Gurer and Ercal [167] also summarize how ALA is involved in ROS generation. Several studies have also reported alterations in antioxidant enzymes activities such as superoxide dismutase, catalase and glutathione peroxidase (GPx) and changes in the concentrations of some antioxidant molecules such as glutathione (GSH) in lead exposed animals and humans [153, 232, 233].

Thus the lead induced oxidative stress has now been proved unequivocally, there are however very few studies which indicate the usefulness of antioxidant either individually or as a complimentary agent during chelation therapy. Gurer and Ercal [167] were the first to start exploring the beneficial role of n-acetylcysteine (NAC) in both in vitro and in vivo models. They investigated the lead extent of oxidative injuries and the benefits of administering NAC orally to rats. They indicated that the antioxidant action of NAC could provide some beneficial effects in lead poisoning treatment independent of chelation [234]. Neal et al. [235] suggested a pro-oxidant effect of ALA and a protective role of NAC in ALA exposed cells.

Another antioxidant, α-Lipoic acid (LA) was also suggested to abate few toxic symptoms of lead [236]. This compound/antioxidant has got the distant advantage over NAC in opposing GSH loss because a small amount of LA will produce the same effects as by a large dose of NAC [167]. It is an excellent antioxidant and function as a co-factor in several multi enzyme complexes. It has got the ability to scavenge some reactive species and can generate other antioxidant from their radical or inactive form and have metal chelating capability [167]. The ability of LA to cross blood brain barrier is an extra advantage. Beneficial effects of LA are independent of its ability to chelate lead but are associated with LA's potential for bolstering thiol antioxidant capacity.

Taurine, a semi essential amino acid has been shown to have a role in maintaining calcium homeostasis, osmoregulation, removal of hypochlorous acid and stabilizing the membranes [237]. Some of the recent data indicate that taurine can act as the direct antioxidant by scavenging ROS and/or as an indirect antioxidant by preventing changes in membrane permeability due to oxidant injury. The zwitterionic nature of taurine gives it high water solubility and low lipophilicity. Consequently compared with carboxylic amino acids, diffusion

through lipophillic membranes is slow for taurine [237]. In the studies conducted by Gurer and Ercal [167], taurine was shown to have beneficial effects in lead induced oxidative stress in Chinese Hamster Ovary (CHO) cells and F344 rats [167]. There was an increased cell survival in taurine treated lead exposed CHO cells while MDA levels were diminished and GSH levels were increased. Similar effects were found in RBC and the brains and livers of lead exposed F-344 rats. In the above study, no change in lead concentrations in the blood, brains, livers and kidneys after taurine treatment (1.2 g/kg/d) was noted.

7. THERAPY

7.1. Chelation treatment

'Chelation' is the formation of a metal ion complex in which the metal ion is associated with a charged or uncharged electron donor referred to as ligand. The ligand may be monodenate, bidenate or multidenate, that is, it may attach or co-ordinate using one or two or more donor atoms. Bidenate ligands form ring structures that include the metal ion and the two-ligand atoms attached to the metal [238]. Their efficacy depends not solely on their affinity for the metal of interest but also on their affinity for endogenous metals, mainly calcium. An ideal chelator should have high solubility in water, resistance to biotransformation, ability to reach site of metal storage, ability to retain chelating ability at the pH of body fluid and property of forming metal complexes that are less toxic than the free metal ion.

Some common chelating agents for the treatment of lead poisoning are listed in Figure 12 along-with their chemical structures. Although most of these chelating agents are effective against lead toxicity but still they do not fulfil the required characteristic for an ideal chelating agent (Figure 13). Their pharmacological and toxicological properties are described below:

7.1.1 Calcium disodium ethylene diamine tetraacetic acid ($CaNa_2EDTA$)

The most commonly used chelating agents that have been the forerunners in chelation therapy belong to the polyaminocarboxylic groups. As the name indicates, these chelators utilize the amino and the carboxylic groups to scavenge lead from the system. In this category, calcium disodium ethylene diamine tetra acetic acid ($CaNa_2EDTA$) is a derivative of ethylene diamine tetra acetic acid (EDTA); a synthetic polyamino-polycarboxylic acid and since 1950s has been one of the main stays for the treatment of childhood lead poisoning [239]. Another member belonging to this family is diethylene triamine pentaacetic acid (DTPA) is a synthetic polyaminocarboxylic acid with properties similar to EDTA [238]. $CaNa_2EDTA$ has the LD_{50} value of 16.4 mmol/kg in mouse [239]. Intravenous administration of this drug results in good absorption but very painful at the injection site. Hence intravenous injection could be given

either by diluting in 5% dextrose or saline [239]. It has also been used extensively as a challenge test for lead [240] in order to help make the decision as to whether chelation therapy should be undertaken especially in children. $CaNa_2EDTA$ is mainly distributed extra-cellularly. One of the major drawbacks with $CaNa_2EDTA$ appears to cause redistribution of lead to the brain [190, 241]. It has been well established that administration of EDTA during pregnancy can result in teratogenic effects especially when administered between days 11 to 14 at doses comparable to humans. 2-3% disodium EDTA when given in feed along with 100 ppm of zinc to pregnant rats from the gestation day 6 through 21 resulted gross congenital malformations which included clubbed legs, micro or anophthalimia, micro or agnathia, cleft palate, fused or missing digits, brain malformation and curly, short or missing tail in the young [242]. The use of EDTA to remove endogenous zinc appeared to offer a mechanism for studying the effects of short-term zinc supplementation at critical periods in pregnant zinc deficient rats. Primary source of lead mobilised by $CaNa_2EDTA$ is bone, with an additional contribution from kidney.$CaNa_2EDTA$ not only increases the urinary excretion of lead but because of its relative lack of specificity the excretion and depletion of such essential metals as Zn, Cu, Fe, Co and Mn is possible. Treatment with Ca disodium EDTA resulted in rapid decrease in plasma zinc concentrations. Administering the sodium salt of EDTA *in vivo* will result in the formation of the calcium salt, which will be excreted. This can result in an increase in the urinary excretion of calcium and hypocalcaemia with the risk of tetany. To overcome this hazard, $CaNa_2EDTA$ was introduced for the treatment of lead poisoning. In this case, the lead-EDTA chelate has the higher stability constant. Thus $CaNa_2EDTA$ chelates the lead in the body fluids, $PbNa_2EDTA$, which is excreted leaving Ca behind. According to a study done by Cory Slechta et al. [190], the rise in brain lead content in response to a single injection of 150 mg/kg of $CaNa_2EDTA$ was observed in rats exposed to 25 and 50 ppm of lead acetate. The failure of Ca disodium EDTA to produce any net loss of lead from brain over the course of the 5-day treatment aroused further concern. 5-days dosing regimen of Ca disodium EDTA after chronic lead exposure reported no decline in brain lead. It should be noted that a 5-days course of treatment is the one most often used clinically to treat elevated lead burden.

7.1.2 D-penicillamine

D-Penicillamine (DPA) is β-β-dimethylcysteine or 3-mercapto-D-valine, a sulfhydryl containing amino acid, is as an antidote for low or mild lead poisoning [243]. It can penetrate cell membranes and then get metabolized. It can be absorbed through the gastro intestinal tract and thus can be administered orally. However, the major toxic effect of DPA is antagonizing pyridoxine and inhibiting pyridoxine dependent enzyme such as transaminases. Other toxic effects include hypersensitive allergic reactions like fever, skin rashes, leucopoenia and thrombocytopenia [244]. Its reported LD_{50} value in mouse by

intraperitoneal route is 2.53mmol /kg. The FDA, for the treatment of Wilson's disease, cystinuria, and severe active rheumatoid arthritis approves penicillamine. Other toxic effects include hypersensitive allergic reactions like fever, skin rashes, leucopoenia and thrombocytopenia. In few reports nephrotoxic effects too have been observed along with penicillin allergic reaction in sensitive individual due to cross reactivity. Prolonged treatment may also lead to anorexia, nausea, vomiting in human. Apart from this, DPA is also a well recognized teratogen and lathyrogen that causes skeletal, palatal, cutaneous and pulmonary abnormalities [243]. The developmental toxicity of DPA is abundant in both human and experimental animals. Since DPA chelated copper, it was hypothesized that the drug might be teratogenic. Literature suggests that the administration of DPA during pregnancy protects the mother from the relapse of Wilson's disease, while it would carry few risks to the fetus [245]. DPA have been tried safely throughout pregnancy in women with Wilson's disease, suggesting that the excessive copper stores improve tolerance [246]. Before the approval of DMSA penicillamine was the only available oral chelator and has been used for the long-term treatment of children with blood lead levels of 20-40 μg/dL [244, 247]. D-penicillamine is a less effective chelator than calcium disodium EDTA and its overall toxicity profile allows it be considered as a third choice for lead toxicity treatment after $CaNa_2EDTA$ and DMSA (Succimer).

7.1.3 Meso 2, 3-dimercaptosuccinic acid (DMSA)

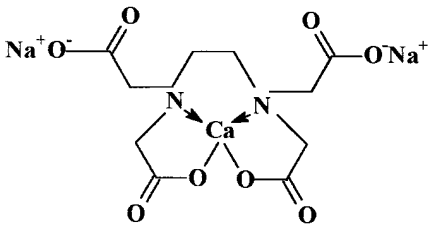
The chemical derivative of dimercaprol, which has gained more and more attention these days, is meso 2, 3-dimercaptosuccinic acid (DMSA). DMSA is an orally active chelating agent that forms stable water-soluble complexes with lead *in vitro*. DMSA contains two sulfhydryl (-SH) groups and has been shown to be an effective chelator of lead, reducing blood lead levels significantly. It has recently been approved by US Food and Drug Administration (US FDA) for the treatment of lead intoxication in children with blood lead levels 45 μg/dL (US Dept. of Health and Human Services). A major advantage of DMSA is that, lead does not appear to be redistributed to the brain and other vital organs after its therapy in rats intoxicated with lead [241, 248]. Another advantage is that DMSA is available in capsule form for oral administration. Animal studies suggest that DMSA is an effective chelator of soft tissue but it is unable to chelate lead from bones [190]. In an interesting study by Ercal et al., lead induced biochemical variables suggestive of oxidative stress responded moderately to treatment with DMSA while, there was a marked reduction in lead concentration from blood, liver and brain [249]. Miller [250] suggested that the protocol for lead toxicity is to identify and remove the environmental exposure and use DMSA 10 mg/kg three times a day for the first five days followed by 14 days at 10 mg/kg twice a day. Studies conducted by Flora et al. [241] shows that DMSA could significantly lower the blood, hepatic and renal burden of lead

after 4 months lead exposure to 1000-ppm lead. $CaNa_2EDTA$ also removed lead from liver and kidneys apart from the long bones. Not-withstanding the significant mobilisation of lead by both DMSA and $CaNa_2EDTA$ individually, there was a significant additive effect as well. The reason is unclear. One possibility is that the kidneys reducing the possibility of lead redistribution to soft tissues [190] might excrete lead mobilised from bone. Combined treatment with DMSA and $CaNa_2EDTA$ is more effective than treatment with individual chelating drugs for the elimination of tissue lead. Of all the organs damaged by lead, the developing brain is particularly vulnerable to lead toxicity. As an important brain region, the hippocampus undergoes number of development and growth in postnatal period [179]. Zhang et al. [251] recently suggested that hippocampus is a main target for lead accumulation and damage. Oxidative damage caused by lead may be implicated in the induction of the cell apoptosis. DMSA for being an antioxidant and a strong lead chelator has been shown to deplete significantly lead from hippocampus leading to recovery in the oxidative stress and apoptosis induced by lead [251].

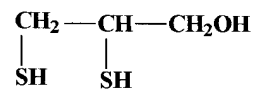
DMSA is not known to cause elevations in the excretion of calcium, zinc or iron, although zinc excretion has increased to 1.8 times base line during treatment. Therefore, in the present circumstances treatment with both $CaNa_2EDTA$ and DMSA seems more adequate but in addition to beneficial effects, the increased renal burden indicates caution; and further investigation in this area is needed before approving this treatment protocol.

7.1.4 Sodium 2, 3-dimercaptopropane-l-sulphonate (DMPS)

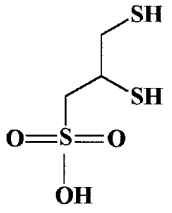
Sodium 2, 3-dimercaptopropane sulfonate (DMPS) is another analogue of BAL and is mainly distributed in extra cellular space and it may enter cells by specific transport mechanism. DMPS is rapidly eliminated from the body through the kidneys. No major adverse effects following DMPS administration in humans or animals have been reported [252]. A pilot study of DMPS in lead poisoned children by Thomas and Chisolm [253] indicates less efficiency than $CaNa_2EDTA$ and DMSA. DMPS appears to be bio-transformed in humans to acyclic and cyclic disulphides. DMPS is distributed both in an extra-cellular and to a small extent an intracellular manner [238]. DMPS is not the appropriate drug as far as lead is concerned. Oral administration of DMPS also did not adversely affects late gestation, parturition or lactation in mature mice and fetal and neonatal development do not appear to be adversely affected. DMPS although known for its antidotal efficacy against mercury and has also been reported to be an effective drug for treating lead and arsenic poisoning [154, 2241, 254].



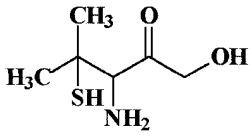
$CaNa_2EDTA$



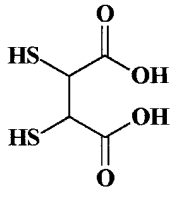
BAL



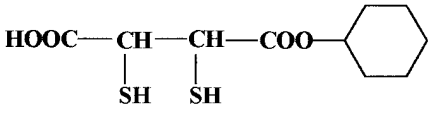
DMPS



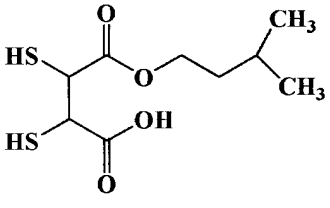
DPA



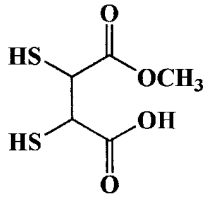
DMSA



MchDMSA



MiADMSA



MmDMSA

Figure 12. Common lead chelators and their chemical structures.

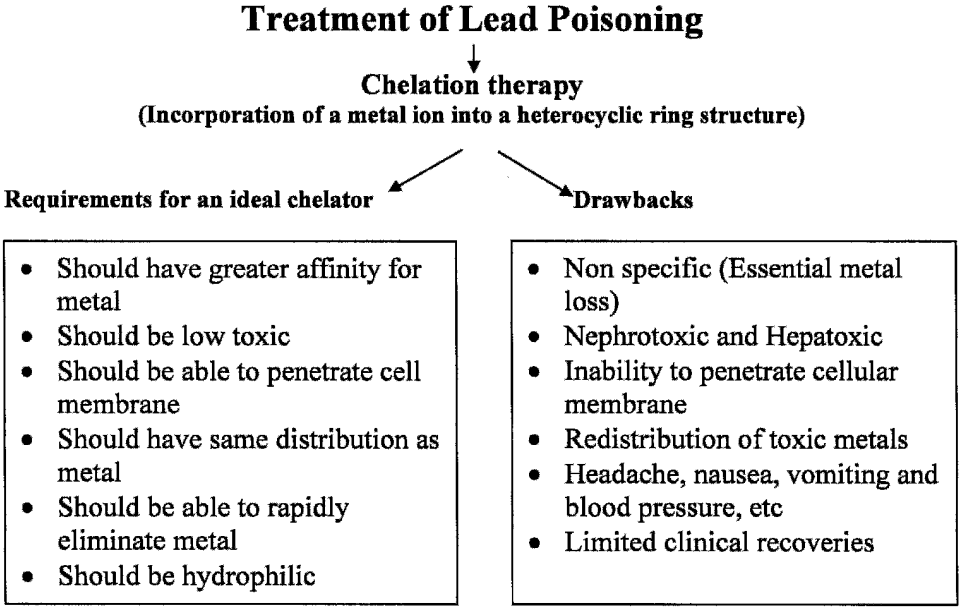


Figure 13. Characteristics of an ideal chelators and drawbacks with the currently available chelating agents

7.2. Limitations of current chelating agents

Most of the currently used chelating agents have serious side effects. $CaNa_2EDTA$ is a general chelating agent that complexes a wide variety of metal ions. It is used clinically [255]. $CaNa_2EDTA$ cannot pass through cellular membranes and therefore its use is restricted to removing metal ions from their complexes in the extra cellular fluid. The principal toxic effects of $CaNa_2EDTA$ are on the kidneys [256, 257]. Renal toxicity may be related to the large amount of chelated metals that pass through the renal tubules in a relatively short period during therapy. Monitoring of zinc status is also recommended during therapy because of massive diuresis of endogenous zinc [258]. Other minor problem with $CaNa_2EDTA$ includes malaise, fatigue, myalgia, anorexia, nausea and vomiting, sneezing, glycosuria, anaemia and hypotension. Intramuscular $CaNa_2EDTA$ is painful too.

The main disadvantage of treatment with the penicillamine as a chelating agent is that it might cause anaphylactic reaction in-patient allergic to penicillin [259]. Prolonged use of penicillamine may induce several cutaneous lesions, dermatomyosites, adverse effects on collagen, dryness etc. The hematological effects include leucopoenia, aplastic anaemia and agranulocytosis. Renal toxicity is usually as reversible proteinuria while, elevation in liver enzymes indicate hepatotoxicity. Children undergoing D-penicillamine therapy should be monitored with blood counts, urinalysis and serum creatinine every 2-4 weeks. In comparison to other chelating drugs, treatment with DMSA and DMPS has got less adverse effects. Few adverse effects include mild and transient elevation of SGPT and SGOT, mild abdominal disturbances and skin reaction possibly of allergic origin. One of the major drawback with the use of DMSA is that it is basically a soft tissue lead mobilizer and thus unable to remove lead from hard tissues like bone. Thus, its use particularly in chronic cases of lead poisoning is limited.

It is thus clear from above that most of the conventional chelators are compromised with many side effects and drawbacks and there is no safe and effective treatment available for lead poisoning. In the recent past some newer strategies were adopted to find a solution to this problem [254]. In the following paragraphs some of these strategies have been discussed in details and summarized in Figure 14.

Among the effective strategies include the effects of oral co-administration of zinc and copper supplementation on the safety and efficacy of $CaNa_2EDTA$ in the treatment of lead poisoning in experimental animals [260-263]. However, it was also suggested that higher dose and prolonged administration of zinc should be avoided [264]. In the second approach, efficacy of some naturally occurring compounds like vitamins and amino acids were tried both as potential chelating agents' and as an adjuvant to conventional chelating agents during experimental lead intoxication. Concomitant oral administrations of thiamine, ascorbic acid, or methionine during experimental lead intoxication has been proved beneficial

[265-269]. A somewhat different class of compounds the esters of dimercaptosuccinic acid has also been investigated as agents to mobilize lead from aged intracellular deposits [270]. The efficacy of S-adenosyl L-methionine in the prophylaxis of lead poisoning has been demonstrated in both human and animals [271-273]. Methionine being a ready source of sulfydryl group would increase the bioavailability of glutathione, which would provide additional complexing, sites for lead poisoning. It appears that dietary nutrients when given during chelation therapy may have a beneficial role in controlling lead poisoning. These strategies which are described below strongly support the theory that they have a major role to play in future approach towards finding a safe, suitable and an effective treatment for lead intoxication.

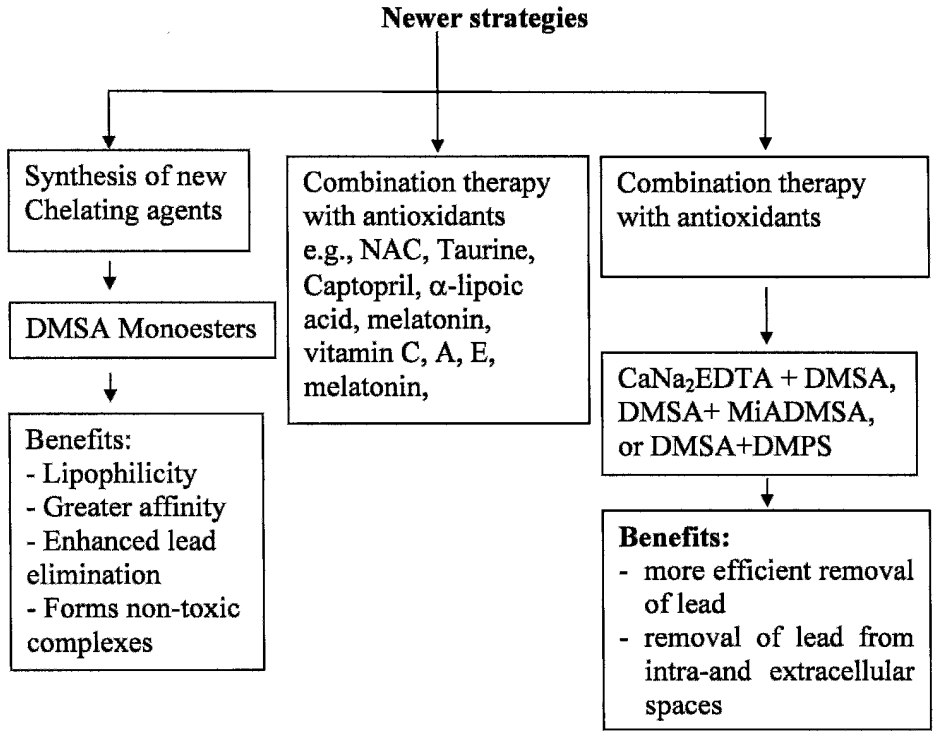


Figure 14. Newer strategies for the treatment of lead poisoning

7.3. Recent developments in the chelation of lead

7.3.1. Synthesis of new chelator

In the early eighties it was shown that some newer complexing agents like DMPS and DMSA were effective against lead poisoning. When compared to BAL these newer chelating agents were of significant lower toxicity and moreover they could be administered orally or intravenously. In addition to their heavy metal chelating properties, these agents have a dithiol group that may act as an oxygen radical scavenger and thus inhibit lipid peroxidation. But still the hydrophilic and lipophobic properties of DMSA do not allow it to cross the cell membrane. It was recently observed that esters of DMSA might be a more effective antidote for lead toxicity

7.3.1.1 Esters of Succimer (DMSA)

A large number of esters of DMSA have been synthesized for achieving optimal effects of chelation compared to DMSA. These esters are mainly the mono and dimethyl esters of DMSA that have been studied experimentally with the aim of enhancing tissue uptake of chelating agents. In order to make the compounds more lipophillic, the carbon chain length of the parent DMSA was increased by controlled esterification with the corresponding alcohol (methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl and hexyl). A large number of esters have been synthesized and are being tried for the treatment of metal poisoning. It has also been reported that these mono and diesters have a better potential in mobilizing lead from the tissues in mice [274]. Singh et al. [275] examined the efficacies of three diesters of DMSA and found that these diesters were effective in reducing the soft organ lead concentrations when compared to BAL. Walker et al. [274] studied the effects of seven different monoalkyl esters of DMSA on the mobilization of lead in mice and observed that after a single parenteral dose of the chelator DMSA there was a 52% reduction in the lead concentrations while with the monoesters the reduction varied from 54% to 75%.

In most of these published reports, it has been observed that the analogues of DMSA were capable of crossing the membranes and were more effective in reducing the lead burden in acute and sub-chronic lead intoxication. These studies have also suggested that the monoesters are more effective in treatment of experimentally induced lead intoxication.

7.3.1.1.1 Monoisoamyl DMSA (MiADMSA)

Among the new chelators, monoisoamyl ester of DMSA (MiADMSA; a C_5 branched chain alkyl monoester of DMSA) has been found to be the more effective than DMSA in reducing gallium arsenide and arsenic toxicity [276, 277]. It is reported that the toxicity of DMSA with LD_{50} of 16 mmol/kg is much lower than the toxicity of MiADMSA with LD_{50} of 3 mmol/kg but lesser than

BAL (1.1 mmole/kg). The interaction of MiADMSA and DMSA with essential metals is same. Mehta and Flora [278] reported for the first time the comparison of different chelating agents (3 amino and 4 thiol chelators) on their role on metal redistribution, hepatotoxicity and oxidative stress in chelating agents induced metallothionein in rats. We suggested that out of all the 7 chelators, MiADMSA and DMSA produced the least oxidative stress and toxicity as compared to other 5 chelators. However, no major reports are available about the toxicity of this metal complexing agent except for its developmental toxicity. No observed adverse effect levels (NOAELs) for maternal and developmental toxicity of MiADMSA were 47.5 mg/kg and 95 mg/kg/day respectively indicating that MiADMSA would not produce developmental toxicity in mice in the absence of maternal toxicity [279]. Bosque et al. [280] reported that administration of MiADMSA through the parenteral route to pregnant mice during organogenesis produced maternal toxicity at a dose of 95 and 195 mg/kg with a significant decrease in the body weight and an increase in the liver weights. They also reported that MiADMSA caused embryo/fetotoxicity at a dose of 190 mg/kg by significantly increasing the embryo lethality and non-significant increase in the skeletal defects. Taubeneck et al. [281] showed that the developmental toxicity of DMSA is mediated mainly through disturbed copper metabolism and this may also be true for MiADMSA. Recently, our group was the first to report the toxicological data of MiADMSA when administered in male and female rats through the oral as well as the intra-peritoneal route (25, 50 and 100 mg/kg for 3 weeks). We observed that there was no major alteration in the heme biosynthesis pathway except for a slight rise in the zinc protoporphyrin levels suggesting mild anemia at the highest dose [282, 283]. The oral route of administration was also seen to be better when compared to the i.p. route based on the histopathological studies of the liver and kidney tissues. MiADMSA was seen to be slightly more toxic in terms of copper loss and some biochemical variable in the hepatic tissue in females as compared to male rats. The studies concluded that the administration of MiADMSA in female rats is confounded with side effects and may require caution during its use. Since administration of chelating agents during pregnancy is always with caution, we studied the effects of MiADMSA administration from day 14 of gestation to day 21 of lactation at different doses through oral and ip routes to examine the maternal and developmental toxicity in the pups. Results suggested that MiADMSA had no effect on length of gestation, litter-size, sex ratio, viability and lactation [284]. No skeletal defects too were observed following the administration of the chelator. However, MiADMSA administration produced some marginal maternal oxidative stress at the higher doses (100mg/kg and 200 mg/kg) based on thiobarbituric acid reactive substances (TBARS) in RBCs and decrease in the δ-aminolevulinic acid dehydratase (ALAD) activity. MiADMSA administration too caused some changes in the essential metal concentration in the soft tissues especially the copper loss in

lactating mothers and pups, which would be of some concern. Apart from copper, changes too were observed in the zinc concentrations in mothers and pups following administration of MiADMSA. The study further suggested that the chelator could be administered during pregnancy as it does not cause any major alteration in the mothers and the developing pups [284]. Since chelating agents are administrable to individuals of all ages, we investigated the effect of MiADMSA administration in different age groups of male rats (young, adult and old rats) based on the fact that whether MiADMSA, a dithiol agent was a pro-oxidant or an antioxidant. Results suggested that MiADMSA administration increased in activity of ALAD in all the age groups and increased blood GSH levels in young rats (Flora et al. unpublished report). MiADMSA also potentate the synthesis of MT in liver and kidneys and GSH levels in liver and brain. Apart from this it also significantly reduced the GSSG levels in tissues. MiADMSA was found to be safe in adult rats followed by young and old rats.

A large number of reports are now available on the therapeutic efficacy of the MiADMSA. Pande et al. [285] found that MiADMSA was effective in prevention and treatment of acute lead intoxication. Walker et al. [274] reported that MiADMSA administration reduced the brain lead concentrations by 75% when compared to 35% with DMSA whereas the ip administration reduced kidney lead levels by 93% while oral administration reduced the kidney lead by 94%.

Despite a few drawbacks/side effects associated with MiADMSA, our studies suggest that MiADMSA may be a future chelating drug owing to its lipophilic character and the absence of any metal redistribution. However, significant copper loss requires further studies. Moderate toxicity after repeated administration of MiADMSA may be reversible after the withdrawal of the chelating agent.

7.3.2 Combination therapy

This is a new trend in chelation therapy that is to use two chelators, which act differently. The idea of using combined treatment is based on the assumption that various chelating agents are likely to mobilise toxic metals from different tissue compartments and therefore better results could be expected [241, 286]. There are four components to treatment identification and elimination of the source of exposure, behavioural changes to diminish non-food-related hand to mouth activity, nutritional counselling to inhibit lead absorption and chelation therapy to increase lead excretion. The choice of treatment modalities depends only in part on the degree of lead poisoning. It is in the interest of all children to be in lead free environment. The very young are at a higher risk than adults. Treatment of lead intoxication has relied primarily on the use of Ca disodium EDTA. A new approach in chelation therapy was therefore to use both EDTA as a bone lead mobilizer and DMSA as a soft tissue mobilizer in combined treatment. This approach of using combined treatment also lead to a better

recoveries in altered lead sensitive biochemical /neurological/ immunological variables and support our earlier assumption that various chelating agents will mobilize lead from different tissue compartments and therefore better results in mobilizing, lead and turnover in the altered clinical variables could be expected. The goal of chelation therapy should be to maximize lead elimination from the body without risking redistribution to soft tissues. We observed that combined administration of DMSA and $CaNa_2EDTA$ against chronic lead poisoning lead to a more pronounced elimination of lead and a better recoveries in altered lead sensitive biochemical variables beside no redistribution of lead to any other organ was noticed [154, 241]. In these experiments we showed that the administration of DMSA before EDTA prevents redistribution of lead to the brain. Furthermore we also found that combined treatment to be more effective in mobilizing lead than monotherapy with either of these chelating agents. However, kidney concentration remained high following combined treatment and some clinical biochemical variables showed minor alterations [241, 287]. After getting some very promising results with the combined administration of DMSA and EDTA we determined if the use of another thiol chelating agent with intracellular distribution like MiADMSA (an analog of DMSA) or DMPS in combination with $CaNa_2EDTA$ might provide a better removal of toxic metal and in providing effective reversal of altered biochemical indices compared to the use of DMSA [154]. The effects of EDTA, DMPS and MiADMSA monotherapy were compared with meso-DMSA plus EDTA or MiADMSA plus EDTA by measuring lead and essential metal concentration in soft tissues, studying various lead sensitive biochemical variables and parameters suggestive of oxidative stress and altered heme synthesis pathway in adult rats. The results of combined treatments with $CaNa_2EDTA$ and the two thiols were more impressive both in terms of recovery in altered biochemical variables (oxidative stress) and reduction of body lead burden. Thus there could be an interesting debate about introducing combined treatment therapy for lead poisoning. The two arguments proposed for promoting this treatment point to the fact that (i) The inefficiency of EDTA to decrease brain lead and the ability of thiol chelators like DMSA for their reported role in promoting soft tissue lead mobilization including brain (ii) addition of MiADMSA to EDTA caused not only higher lead elimination but also better recovery in altered biochemical variables. The present study thus represents a systematic approach to the development of a new therapeutic protocol for the treatment of lead intoxication. Besunder et al. [288] also recommended the administration of EDTA and DMSA to, children hospitalized for chelation therapy instead of monotherapy with either agent. Beside the use of the two different chelators for the combined therapy, number of studies have been reported where a co-administration of a dietary nutrients like a vitamins e.g. thiamine [263, 265], an essential metal viz. zinc [261, 263, 264] or an amino acid like methionine [269] with a chelating

agent lead to many beneficial effects like providing better clinical recoveries as well as mobilization of lead.

As evident from the above, the most important problem concerning the medical use of chelating agent is their low therapeutic range, which is mainly due to the inherent toxicity of the chelators itself. Chelation is not specific to toxic ions and is causing disturbances of all biological processes depending on a physiological equilibrium of ions. In general, chelators with the intra-cellular activity are more toxic and have a lower therapeutic range than those distributed only in the extra cellular spaces.

7.3.3 Role of dietary nutrients during chelation of lead

Among the various strategies which have been suggested to minimise the numerous problems, the use of an 'adjuvant' viz. essential metals, vitamins and amino acids, etc is an interesting one [260]. The defense of biological system against damage caused by activated oxygen involves a battery of interrelated protective agencies, the micronutrients, which have come to be regarded as antioxidant nutrients, lie functionally at the heart of this protective mechanism and include vitamins such as α-tocopherol, ascorbic acid etc. Antioxidants when given either alone or in combination with a chelating agent proved to be more effective in mobilizing lead from soft as well as hard tissue. It is now well known that lead causes their toxicity by the involvement of reactive oxygen species (ROS). Lead binds to biological molecules and produce different free radicals that in turn attack the building blocks of the biological systems. Deficiency of several essential nutrients namely vitamins and essential elements, has been shown to exacerbate the toxic effects of metals, and supplementation of such nutrients ameliorates the toxicity. In addition to the role of micronutrients in modifying metal toxicity, these nutritional components can also act as complimentary chelating agents (adjuvant) increasing the efficacy of a known chelator, or by acting independently. In the following paragraph, we discuss some of the micronutrients/essential metal/vitamins/antioxidants, which could be useful when administered during chelation therapy.

7.3.3.1 Essential metals

We have discussed above that micronutrients have some role in preventing toxic metal absorption and could also be co-administered during chelation therapy as maintenance of essential metal status during chelation treatment and could serve dual purpose; i) to prevent possible essential metal deficiency syndrome and ii) to accelerate lead elimination due to their own antagonistic/biochemical/pharmacological effects [289, 290]. Beneficial role of zinc supplementation during chelation therapy of lead has been reported [264]. A more effective removal of hepatic and renal lead and recoveries in the lead sensitive biochemical indices may offer an answer to the problem raised with Ca disodium EDTA ($CaNa_2EDTA$) therapy. We however, cautioned the excess and

prolonged use of zinc, which may not allow the chelator to bind lead and rather zinc in preference to lead [261]. Thomas and Chisolm [253] found that oral supplementation of zinc and copper salts during the course of $CaNa_2EDTA$ treatment did not alter the urinary excretion of lead or zinc but reduced the fall in plasma zinc concentration [263]. The oral supplementation of zinc during chelation therapy has been found to be beneficial in a patient of plumbism. Simultaneous copper supplementation although have very limited role during chelation of lead [262].

7.3.3.2 Vitamins

Thiamine as a complementary therapeutic agent or an `adjuvant' to conventional metal chelating agent has been tried [260, 291]. Thiamine administration concomitantly with $CaNa_2EDTA$ enhanced the efficacy of chelator to potentiate urinary lead excretion, to reduce tissue lead including brain lead and restore lead induced biochemical alterations [265, 292]. Thus, thiamine might be utilized to increase the passage of chelating agents through blood -brain barrier or its effect to decorporate lead from the brain bioligand complexes [293]. Thiamine might also be participating in chelation as it contains a pyrimidine ring and a thiazole nucleus. The -OH group of the side chain and S atom of the thizole nucleus from 2 moles of thiamine HCl may participate in the chelation of lead (Figure 15) [294]. Further N atoms or NH_2 groups of the pyrimidine nucleus in the thiamine molecule might also be playing a role in chelation of lead. Thiamine supplementations during chelation only slightly augmented lead decorporation but the depletion of brain lead was significant following treatment with thiamine- DMSA [293, 294]. These observations might be significant, as some of the chelators including $CaNa_2EDTA$ have been shown to be ineffective in removing lead from the brain. Co-administration of vitamin C and thiamine greatly enhanced the efficacy of chelating agents to increase urinary lead excretion, to reduce tissue lead concentration including brain, supporting the view that ascorbic acid acts as a detoxifying agent by forming poorly ionised but soluble complex with lead (Figure 15) [212, 268]. Vitamin C is a low molecular mass antioxidant that interacts directly with the oxidizing radicals and protects the cells from reactive oxygen species [295]. Vitamin C scavenges the aqueous reactive oxygen species (ROS) by very rapid electron transfer that thus inhibits lipid peroxidation [295].

The protective mechanism of vitamin E against lead toxicity could be attributed to its antioxidant property or its location in the cell membrane and its ability to stabilize membrane by interacting with unsaturated fatty acid chain. Flora et al [296] reported that administration of Vitamin C or vitamin E when given in combination with succimer or its monoisoamyl derivative (MiADMSA) produced profound recoveries in sub-chronically lead exposed rat. Although, the group suggest that vitamin C was better in providing clinical recoveries and Vitamin E was equally efficient in decreasing the lead burden from the tissues.

7.3.3.3 Antioxidants

N-Acetylcysteine (NAC) is a thiol-containing antioxidant that has been used to mitigate various conditions of oxidative stress. Its antioxidant action is believed to originate from its ability to stimulate GSH synthesis, therefore maintaining intracellular GSH levels and scavenging reactive oxygen species (ROS) [167, 297]. Besides the antioxidant potential, NAC also has some chelating properties against lead. Pande et al. [285] suggested that NAC could be used both as preventive as well as a therapeutic agent along with MiADMSA/DMSA in the prevention and treatment of lead intoxication in rats. They also reported that simultaneous administration of NAC with DMSA reversed the altered ALAD and TBARS levels, increased the reduced glutathione levels and decreased the lead levels, apart from this the study too highlighted the favorable response of NAC in post-exposure treatment along with succimer [285]. A recent report suggested that co-administration of NAC along with succimer in sub-chronically lead exposed rats, reduced oxidative stress significantly by lowering the TBARS levels, oxidized glutathione levels along with the decrease in the lead burden on the soft tissues especially the brain [276].

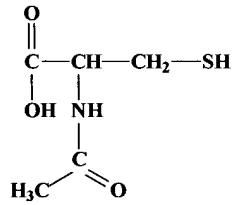
Melatonin (N-acetyl-5-methoxy triptamine), is a hormonal product of the pineal gland that plays many roles within the body including control of reproductive functions, modulation of immune system activity, and limitation of tumorigenesis and effective inhibition of oxidative stress [298]. One major function of melatonin is to scavenge radicals formed in oxygen metabolism, thereby potentially protecting against free radical induced damage to DNA, proteins and membranes [298]. It has been shown that melatonin stimulates the antioxidative enzyme GPx in the brain, thus providing indirect protection against free radical attack [299]. Therapeutic efficacy of melatonin either individually or in combination with succimer (DMSA) was recently studied by us in rats [300]. We found very little role of melatonin in the mobilization of lead but it provided significant protection to lead induced oxidative stress in tissues of lead exposed rats. The therapeutic efficacy of melatonin has been reported to be better than other antioxidants like glutathione and vitamin E.

α-Lipoic acid (LA) is a naturally occurring antioxidant and is able to abate some of the toxic effects of lead [236]. It functions as a cofactor in several multienzyme complexes. Its reduced form, dihydrolipoic acid (DHLA), has two free sulfhydryl groups and the two forms LA/DHLA possess a great antioxidant potential. Both LA and DHLA (i) have the ability to scavenge some reactive species (ii) can regenerate other antioxidants (i.e. vitamins E and C and GSH) from their radical or inactive forms, and (iii) have metal chelating activity. Lipoic acid also have an advantage over NAC in opposing GSH loss, since LA is effective in a micro molar range while mill molar NAC is needed for a similar effect [301]. The capability of LA to cross the blood brain barrier is an extra

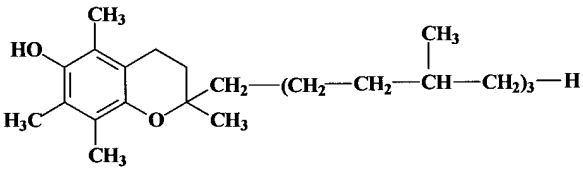
advantage because the brain is an important target in lead poisoning. We provided experimental evidence of beneficial effect of combined LA-succimers administration for treatment of sub-acute lead intoxication in rats. Administration of LA with DMSA or MiADMSA was most effective in reducing lead induced oxidative stress in brain compared to monotherapy [302]. LA administration however, showed no chelating properties in decreasing lead burden from blood and soft tissues except interestingly, more pronounced decrease in brain lead concentration in animals LA plus thiol chelators compared to the effect of thiol chelators. The mechanism for the beneficial effect of LA could be attributed to its ability to scavenge some reactive species, to regenerate other antioxidant and also to some extend its moderate chelating property.

An antioxidant mechanisms rather than a chelating activity, seems to underlie the observed effects of taurine against lead-induced oxidative stress. We recently described the dose dependent effect of taurine, either alone or in combination with meso 2, 3-dimercaptosuccinic acid (DMSA) in the treatment of sub-chronic lead intoxication in male rats [303]. The results suggested beneficial role of taurine when administered along-with DMSA in providing effective reversal of number of lead sensitive biochemical variables in general, and parameters of oxidative stress in particular, compared to their individual effects. We noted significant effect of taurine when co-administered with DMSA, in depleting blood and brain lead [303]. It is known that highest concentration of taurine is in brain and heart. Perhaps this in part might explain the significant elimination of lead from the brain tissues. Taurine can act as a direct antioxidant by scavenging reactive oxygen species or as an indirect antioxidant by preventing changes in membrane permeability due to oxidant injury. This is an interesting and significant observation, which requires further exploration. Taurine might be improving the antioxidant defense system via inhibiting the lipid peroxidate process thereby mitigating the GSH consumption The results thus suggest the beneficial role of taurine when administered along with a thiol chelator, but still it remains to be seen if- (i) taurine is a better antioxidant than other available antioxidants in providing significant clinical recovery; (ii) a dose dependent study using a higher dose of taurine need to be attempted; and (iii) the exact mechanism of action of taurine needs to be elucidated.

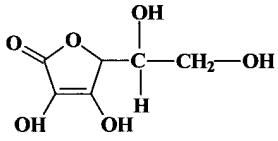
Use of antioxidants thus brings another novel option to the therapy i.e. the possibility of therapeutic intervention without removing the patient from the source of lead. Antioxidants are recognized as safe molecules and may be given to subjects with low level lead concentrations in their blood even when it is not possible to remove them from exposure to lead. The chemical structures of various antioxidants are given in Figure 15.



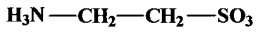
N-acetyl cysteine (NAC)



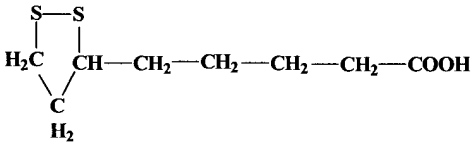
α-Tocopherol (Vitamin E)



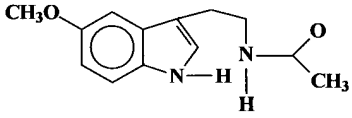
Ascorbic acid (Vitamin C)



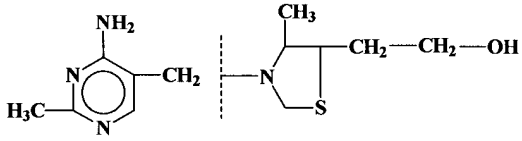
Taurine (2-aminoethane sulfonic acid)



α-Lipoic acid



Melatonin



Pyrimidine nucleus Thiazole nucleus

Thiamine

Figure 15. Chemical structures of antioxidants known for their protective efficacy against lead

7.3.3.4 Role of herbal extracts

The clinical importance of the herbal drugs has recently received considerable attention. Their role although, have mainly been limited to act as an antioxidant and to provide better recovery in the altered biochemical variables. None of them so far have been found to have property to chelate lead. Thus, they have been tried mainly as supplementary agent during chelation of lead. As numbers of synthetic antioxidants have been shown to have side effects [304, 305], thus, there has been upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue injury caused due to lead exposure [306]. Several naturally occurring dietary or non-dietary constituents as well as part of several species of edible plants with pharmacological activity influence the antioxidant enzymes and provide protection against free radical induced damage. *Centella asiatica* (Umbelliferae) syn *Hydrocotyl asiatica* has been mentioned in ancient Indian literature for its intelligence property (Charak Samhita). It is commonly known as Indian Pennywort in English and Brahmi in Hindi. In a recent study, we observed that combined treatment with a thiol chelator (DMSA and MiADMSA) and the natural antioxidant, *Centella asiatica* proved to be beneficial in the recovery of lead induced oxidative stress including the levels of biogenic amines and body lead burden as compared with the monotherapy with the chelating agent [307]. The plant *Centella asiatica* contains asiaticoside, thakunoside, thankunic acid, asiatic acid, brahmoside, brahminoside, brahmic acid, iso-brahmic acid, centoic acid and centilic acid. Further, administration of *C. asiatica* during chelation provided more pronounced effects particularly in the recovery of oxidative stress parameters suggesting that with the removal of lead from the target tissue, this antioxidant provides effective reversal in the altered parameters indicative of oxidative stress. The major hypothesis behind using combination therapy of chelating agents with naturally occurring antioxidant like *C. asiatica* is that: (i) *C. asiatica* is reported to cross the blood brain barrier and recover the altered neurotransmitters levels [308] and because of its ability to restore impaired pro-oxidant/ antioxidant balance will accelerate clinical/ biochemical recoveries, and (ii) MiADMSA, because of its lipophilic nature, will have intracellular access and hence would be more effective in the mobilization of metal out from the body.

Crude extracts of leaves of Indian spinach (Beta vulgaris L. var. beghalensis Hort.) was observed to modify significantly the cytotoxic effects of lead in vivo [309]. These protective effects were attributed to antioxidant properties of number of components of this herbal extracts like vitamins, ascorbic acid, polyphenol and fibres. Chaurasia et al. [310] reported beneficial effects of Withania somnifera, a subtropical under-shrub in protecting mice against lead induced oxidative damage. There are only few such encouraging reports which are available in literature; there are still no clinical reports available on the effect of these herbal extracts on lead toxicity. Thus detailed studies are needed to

study the effects of these plant extracts and to know their mechanism action in the body.

8. CONCLUSION

Lead poisoning is an old and a significant public health problem throughout the world. Although the incidence if occupational and adult lead poisoning has declined in the recent past, the problem still exists. It often goes unrecognized for long periods because of low index of suspicion compounded by incomplete surveillance of risk population. Toxicity correlate with blood lead concentration and progresses from biochemical and sub-clinical abnormalities at levels around 10 μg/dL to coma and death at levels over 100 μg/dL. Blood lead levels below 70 μg/dL can result in damage to the central nervous system, kidneys and hematopoietic system. Lead toxicity is also associated with a two to three point decrease in IQ test scores for every increase in 10 μg/dL in the blood lead level. Elevated blood lead is also associated with neurodevelopment abnormalities including attention deficit disorders, behavioral disturbances, learning disabilities and deficits in fine and gross development. Studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system via depleting glutathione, inhibiting many other enzymes and essential metals needed for antioxidant enzyme activities. Although guidelines for the management of childhood lead poisoning were released by the Center for Disease Control in 1985 and 1991 we are still far away from having a safe, specific and effective chelating agent for the treatment of lead poisoning. Besides, still further knowledge is needed in several basic research areas within the field of *in vivo* chelation of metals and call for studies on (a) Molecular mechanism of action of clinically important chelators, (b) Intracellular and extra cellular chelation in relation to mobilization of aged metal deposits and the possible redistribution of toxic metal to sensitive organs as the brain, (c) Effect of metal chelators on biokinetics during continued exposure to metal, especially possible enhancement or reduction of intestinal metal uptake, (d) Combined chelation with lipophilic and hydrophilic chelators, which presently has a minimal clinical role, (e) Use of antioxidants, micronutrients or vitamins as complimentary agents or antagonists (f) Minimization of the mobilization of essential trace elements during long-term chelation, and (f) Fetotoxic and teratogenic effects of chelators.

Acknowledgment: Authors thank Director, DRDE, Gwalior, India, for his support and Mr. Tapan Das for the secretarial assistance.

REFERENCES

[1] Centers for Disease Control and Prevention (CDC). Atlanta, GA: US Dept of Health and Human Services, 1991.
[2] F. Coulston, N.Y. Albany and F. Korte (eds.), Lead, Georg Thieme Publishers, Stuttgart, 1975, pp 6- 75.
[3] J.J. Breen and C.R. Stroup (eds.), Lead Poisoning: Exposure, Abatement, Regulation, Boca Raton, FL, CRC Press, 1995, pp. 3-12
[4] M.D. McElvaine, E.G. DeUngria and T.D. Matte, Pediat., (1992) 740.
[5] U.S. Environmental Protection Agency (EPA), 1999, 747, R99-002.
[6] U.S. Consumer Product Safety Commission, (1990), Washington D.C.: U.S. Government Printing Office.
[7] B.P. Lanphear, R. Hornung and M. Ho, Pub. Health Rep., 120 (2005) 305.
[8] D.C. Bellinger, Pediatr. 113 (2004) 1016
[9] H. Needleman (ed.), Human Lead Exposure, CRC Press, New York, 1992.
[10] F.R. Moreira and J.C. Moreira, Rev Panam Salud Publication, 15 (2004) 119.
[11] W. Galke, S. Clark, P. McLaine, R. Bornschein, J. Wilson, P. Succop, S. Roda, J. Breysse, D. Jacobs, J. Grote, W. Menrath, S. Dixon, M. Chen and R. Buncher, Environ. Res., 98 (2005) 315.
[12] National Academy of Sciences/National Research Council. Measuring Lead Exposure in Infants, Children, and other Sensitive Populations. Washington, D.C.: World Bank, August, 1996.
[13] M. Markowitz, Pediat. Rev., 21 (2000) 327.
[14] M.B. Rabinowitz, C.W. Wetherill and J.D. Kopple, Am. J. Clin. Nutr., 33 (1980) 1784.
[15] Center for Disease Control (CDC), Atlanta, GA, USA, 1991
[16] Guidelines for drinking water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. Geneva, World Health Organization, 1996; p 254
[17] R. J. Russell, Lancet, 2 (1989) 669.
[18] A.T. Philip and B. Garson, Clin. Lab Med., 14 (1994) 423.
[19] I.A.S. AI-Saleh, Med. Res. Rev., 14 (1994) 415.
[20] H.L. Needleman, Am. J. Public Health, 88 (1998) 1871.
[21] E.K. Silbergeld, Environ. Health Perspect., 91 (1991) 6
[22] D. Rosner and G. Markowitz, Am. J. Public Health, 75 (1980) 344.
[23] M.B. Rabinowitz and M.L. Needleman, Lancet 1 (1983) 63.
[24] Department of Housing and Urban Development (1995), Washington, DC,
[25] C. Staes, T. Matte and C.G. Copley, Am. J. Epidemiol., 139 (1994) 1016.
[26] H.L. Needleman, Environ. Res., 74 (1997) 95.
[27] B.P. Lanphear, T.D. Matte and J. Rogers, Environ. Res., 79 (1998) 51.
[28] W. Galke, S. Clark, J. Wilson, D. Jacobs, P. Succop, S. Dixon, B. Bornschein, P. McLaine and M. Chem, Environ. Res., 86 (2001) 149.
[29] Agency for Toxic Substances and Disease Registry (ATSDR), 2000.
[30] K.P. Farrell, M.C. Brophy and J.J. Chisholm Jr., Am. J. Public Hlth., 88 (1998) 1837.
[31] B.L. Gulson, Env. Health Perspectives, 104 (1996) 306.
[32] B.L. Gulson, J.J. Davis, K.J. Mizon, M.J. Korsch, and J. Bawdensmith, Sci. Total Environ., 166 (1995) 245.
[33] J.E. Ericson and E.J. Gonzalez, Environ. Res., 92 (2003) 21.
[34] J.O. Muse, C.N. Carducci, J.D. Stripeikis, M.B. Tudino and F.M. Fernandez, Environ. Pollut., 2006, In Press.
[35] H. Deng, Z.H. Ye and M.H. Wong, Environ. Pollut., 2006, In Press.
[36] M.E. Finkelstein, R.H. Gwiazda and D.R. Smith, Environ. Sci. Technol., 37 (2003) 3256.

[37] M.S. Baptista, M.T. Vasconcelos, J.C. Paulo Cabral, M.F. Carmo Freitas and A.M. Pacheco, J. Environ. Monit., 8 (2006) 147.
[38] K.C. Staudinger and V.S. Roth, Am. Fam. Phy., 57 (1998) 731.
[39] P.J. Landrigan, E.K. Silbergeld, J.R. Froines and R.M. Pfeiffer, Am. J. Pub. Health, 80 (1990) 907.
[40] C. Zenz, O.B. Dickerson, Jr. and E. P. Horvath, (eds.), Occupational Medicine. 3rd ed. St. Louis, Mosby, 1994, pp.506-541.
[41] J LaDou, (ed.), Occupational Medicine, Conn. Appleton & Lange, Norwalk, 1990, pp 306-10.
[42] J.D. Kaufman, J. Burt and B. Silverstein, Am. J. Indus. Med., 26 (1994) 703.
[43] W. N. Rom. (ed.), Environmental and Occupational Medicine. 2nd ed., Brown, Boston: Little Rock, 1992, pp735-58.
[44] L. Rosenstock and M.R. Cullen MR, (eds.), Textbook of Occupational and Environmental Medicine, Saunders, Philadelphia, 1994, pp745-54.
[45] B.L. Gulson, C.W. Jameson and K.R. Mahaffey, J. Lab. Clin. Med., 130 (1997) 51.
[46] D.G. Hodgkins, T.G. Rogins and D.L. Hinkamp, Br. J. Ind. Med., 49 (1991) 241.
[47] J.L. Stauber, T.M. Florence and B.L. Gulson, Sci. Total Environ., 145 (1994) 143.
[48] A.T. Philip and B. Gerson, J. Clin. Lab. Med., 14 (1994) 423.
[49] A.B. Bruner, A. Joffe, A.K. Duggan, J.F. Casella and J. Brandt, Lancet, 348 (1996) 992.
[50] S.G. McGregor and C. Ani, J. Nutr., 131 (2001) 649S.
[51] E.J. O'Flaherty, Toxicol. Appl. Pharmacol., 131 (1995) 297.
[52] Y. Cheng, W.C Willett, J. Schwartz, D. Sparrow, S. Weiss and H. Hu, Am. J. Epidemiol., 147 (1998) 1162.
[53] K.M. Cake, R.J. Bowins and C. Vaillancourt. Am. J. Ind. Med., 29 (1996) 440.
[54] A.J.A. AI-Modhefer, M.W.B. Bradbury and T.J.B. Simmons Clin. Sci., 81 (1991) 98.
[55] S.R. Grobler, F.S. Theunissen, T.J. Kotze. Arch. Oral Biol., 45 (2000) 607.
[56] J. David, "The Health Effects of Lead on the Human Body" Lead Perspectives, 1996, pp.10.
[57] B.L. Gulson, J.G. Pounds, P. Mushak, B.J. Thomas, B. Gray and M.J. Korsch, J. Lab. Clin. Med., 134 (1999) 631.
[58] H.G. Abadin, B.F. Hibbs and H.R. Pohl, Toxicol. Ind. Health, 15 (1997) 1.
[59] B.J. Lagerkuist, S. Ekesrydh and Y. Englyst, Am. J. Public Health, 86 (1996), 1247.
[60] M. Schumacher, M. Hernandez and J.L. Domingo Trace Elem. Elec., 13 (1996) 177.
[61] M.T. Antonio, I. Corpas and M.L. Leret, Toxicol. Lett., 104 (1999) 1.
[62] P.S. Spencer, H.H. Schaumburg and A.C. Ludolph (eds.) Experimental and Clinical Neurotoxicology. 2nd edition, Oxford University Press, New York; 2000. p. 708
[63] Y. Finkelstein, M. Markowitz and J. Rosen, Brain Research Reviews, 27 (1998) 168.
[64] A.L. Mendelsohn, B.P. Dreyer, A.H. Fierman, C.M. Rosen, L.A. Legano and H.A. Kruger, Pediatrics, 101 (1998) E10.
[65] E.K. Jaffe, J. Bioenerg. Biomem., 126 (1995) 169–179.
[66] V.A. Fitsanakis and M. Aschner. Toxicol. Appl. Pharmacol., 204 (2005) 343.
[67] M. Yasui, N. Pages, and R. Deloncle (eds.), Mineral and Metal Neurotoxicology, CRC Press: New York, 1997, 262.
[68] S. Tong. JAMA, 280 (1998)1915.
[69] B.P. Lanphear, K. Dietrich, P. Auinger and C. Cox, Public Health Rep., 115 (2000) 521.
[70] L.A. Bergdahl, L. Gerhardsson, A. Schutz, R.J. Desnick, J.G. Wetmur, S. Sassa and S. Skerfying, Pharmacol. Toxicol., 81 (1997) 153.
[71] S. Piomelli, Pediatr. Clin. N. Am., 49 (2002) 1285.
[72] E.K. Jaffe, Acta Crystallography. Biol. Crystallography, 56 (2000)115.
[73] J.G. Wetmur, Environ. Health Perspect., 102 (1994) 215.

[74] R.F. Labbe, H.J. Vreman and D.K. Stevenson, Clin. Chem., 45 (1999) 2060.
[75] P. Grandjean, Br. J. Indus. Med., 48 (1991) 254.
[76] C.R. Scriver, A.L. Beaudet, W.S. Sly and D. Valle (eds.) "The Metabolic and Molecular Bases of Inherited Disease (7th edition)." McGraw-Hill: New York, 2001, p 2103-2159
[77] B.S. Schwartz, B.K. Lee, W. Stewart, K.D. Ahn, K. Springer and K. Kelsey, Am. J. Epidemiol., 142 (1995) 738.
[78] Y. Morita, T. Sakai, S. Araki, T. Araki and Y. Masuyama, Sangyo Igaku, 35 (1993) 112 (in Japanese).
[79] D.A. Cory-Slechta, Neurotoxicol. Teratol., 17 (1995) 219.
[80] P.B. Hammond and K.N. Dietrich, Rev. Environ. Contam. Toxicol., 115 (1990) 91.
[81] V. Batuman, J.K. Maesaka and B. Haddad, N. Engl. J. Med., 304 (1991) 520.
[82] G.D. Nuyts, E. Van Vlem and J. Thys, Lancet 346 (1995) 7.
[83] A.I. Sanchez-Fructuoso, A. Torralbo and M. Arroyo. Nephrol. Dial. Transplant, 11 (1996) 1775.
[84] A. Vyskocil, V. Semecky, Z. Fiala, M. Cizkova and C. Viau, J. Appl. Toxicol., 15 (1995) 257.
[85] M. Damek-Poprawa, and. Sawicka-Kapusta, Environ. Res., 96 (2004) 72.
[86] A. Bernard and R.R. Lauwerys. Crit. Rev. Toxicol., 21 (1991) 373.
[87] P.E. McKinney, Clin. Toxicol., 38 (2000) 435.
[88] S.M. Pueschel, J.G. Linakis and A.C. Anderson (Eds.). Lead Poisoning in Children. Baltimore, MD, P.H. Brookes; 1996. p. 75–96.
[89] S. Tong, P. Baghurst, A. McMichael, M. Sawyer, and J. Mudge, Br. Med., J, 312 (1996), 1569
[90] D.C. Bellinger, Neurotoxicol. Teratol.. 22 (2000) 133.
[91] J. Bressler, K.A. Kim, T. Chakraborti and G. Goldstein, Neurochem. Res., 24 (1999) 595.
[92] L.L. Brown, J.S. Schneider and T.I. Lidsky. Curr. Opin. Neurobiology, 7 (1997) 157.
[93] D.E. Glotzer, Pediatric Ann., 23 (1994) 607.
[94] H.L. Needleman, and L.A. Gatsonis, J. Am. Med. Assoc., 263 (1990) 673.
[95] R. Nevin, Environ. Res., 83 (2000), 1.
[96] G.W. Goldstein, Environ. Health Perspect., 89 (1990) 91.
[97] X.M. Shen, Environ. Res., 79 (1998) 1.
[98] C. Ferguson, M. Kern and G. Audesirk. Neurotoxicol., 2000; 21: 365–78.
[99] H. VonKoss Krowchuck, Ann. Rev. Nursing Res., 13 (1995) 87.
[100] R. Bettaiya, P.R. Yallapragada, E. Hall and S. Rajanna, Ecotoxicol. Environ. Safety, 33 (1996)157.
[101] V.A. Dyatlov, A.V. Platoshin and D.A. Lawrence, Neurotoxicol., 19 (1998) 283.
[102] B. Dabrowska-Bouta, G. Sulkowski, G. Bartosz, M. Walski and U. Rafalowska, J. Mol. Neurosci., 13 (1999) 127.
[103] B. Dabrowska-Bouta, G. Sulkowski, M. Walski, L. Struzynska, A. Lenkiewicz, and U. Rafalowska, Exp. Toxicol. Pathol., 52 (2000) 257.
[104] R.A. Gordon, Arch. Pediat. Adoles. Med., 152 (1998)1100.
[105] M.K. Nihei, J.L. McGlothan, C.D. Toscano and T.R. Guilarte, Neurosci. Lett., 298 (2001) 212.
[106] B. Quintanilla-Vega, D.R. Smith, M.W. Kahng, J.M. Hernadez, A. Albores and B.A. Fowler, Chem. Biol. Interact., 98 (1995) 193.
[107] C.M. Bouton, L.P. Frelin, C.E. Forde, H.A. Godwin and J. Pevsner, J. Neurochem., 76 (2001) 1724.
[108] E.G. Moreira, I. Vassilieff and V.S. Vassilieff, Neurotoxicol. Teratol., 23 (2001) 489.

[109] G.T. Ramesh, S.K. Manna, B.B. Aggarwal and A.L. Jadhav, J. Neurosci., 21 (2001) 3369.
[110] G. Sulkowski, B. Dabrowska-Bouta, J. Waskiewicz and U. Rafalowska, Folia Neuropathol., 37 (1999) 205.
[111] M. Correa, A.F. Roig-Navarro and C.M.G. Aragon, Life Sci., 74 (2004) 2009
[112] C.L. Zuch, D.J. O'Mara and D.A. Cory-Slechta, Toxicol. App. Pharmacol., 150 (1998) 174.
[113] D.A. Cory-Slechta, D.V. Widzowski and M.J. Pokora, Neurotoxicol., 14 (1993) 105.
[114] T. Ma, H.H. Chen and I.K. Ho, Toxicol. Lett., 105 (1999) 111.
[115] K.N. Lindgren, V.L. Masten and D.P. Ford, Occup. Environ. Med., 53 (1996) 472.
[116] M. Payton, K.M. Riggs and A.I. Spiro, Neurotoxicol. Teratol., 20 (1998) 19.
[117] K.D. Gill, V. Gupta and R. Sandhir, Cell. Biochem. Funct., 21 (2003) 345.
[118] C.D. Toscano and F.A. Schann, Ann. NY Acad. Sci., 919 (2000) 309.
[119] J.H. Byrne and E.R. Kandel, J. Neurosci., 6 (1996) 425.
[120] V.A. Dyatlov and D.A. Lawrence, Brain Behav. Immun., 16 (2002) 477.
[121] M.J. McCabe, K.P. Singh Jr. and J.J. Reiner Jr., Toxicol. App. Pharmacol., 177 (2001) 219.
[122] P. Boscolo, M. D. Gioacchino, E. Sabbioni, F. Benvenuti, P. Conti, M. Reale, P. Bavazzano and G. Giuliano, Int. Arch. Occup. Environ. Health, 72 (1999) 26.
[123] M.J. McCabe and K.P. Singh Jr., J.J. Reiner Jr., Toxicol., 139 (1999) 255.
[124] P. Apostoli, J. Biol. Sci. Res., 19 (1998) 575.
[125] K.P. Mishra, V.K. Singh, R. Rani, V.S. Yadav, V. Chandran, S.P. Srivastava and P.K. Seth, Toxicol., 188 (2003) 1.
[126] A.H. Alomran and M.N. Shleanmoon, J. Biol. Sci. Res., 19 (1988) 575.
[127] I. Kumber, M.D. Stonard and D.A. Gidlow, Int. Arch. Occup. Environ. Health, 57 (1986) 117.
[128] R.R. Dietert, J.E. Lee, I. Hussain and M. Piepenbrink, Toxicol. Appl. Pharmacol., 98 (2004) 86.
[129] M.J.J. Ronis, T.M. Bedger and S.J. Shema, J. Toxicol. Environ. Health, 54 (1998) 101.
[130] M.J.J. Ronis, J. Gandy and T.M. Bedger, J. Toxicol. Environ. Health, 54 (1998) 77.
[131] J.W. Mitchell, J. Occup. Med., 29 (1987) 397.
[132] P.J. Li, Y.Z. Sheng, Q.Y. Wang, L.Y Gu and Y.L. Wang, Biomed. Environ. Sci., 13 (2000) 85
[133] R.K .Dearth, J.K. Hiney, V.K. Srivastava, S.B. Burdick, G.R. Bratton and W.L. Dees, Toxicology 16 (2002) 343.
[134] B.H. Alexander, H. Checkoway and C. Van Netten, Occup. Environ. Med., 53, (1996) 411.
[135] S. Lin, S. Hwang and E.G. Marshall, Ann Epidemiol., 6 (1996) 201.
[136] M.J. Kosnett, C.E. Becker, J.D. Osterloh, T.J. Kelly and D.J. Pasta, JAMA 271 (1994) 197.
[137] M. Rabinowitz, Environ. Health Perspect, 91 (1991) 33
[138] J.G. Pound, G.J. Long and J.F. Rosen, Environ. Health Perspect., 91 (1991) 17.
[139] H.L. Needleman, J.A. Riess, M.J. Tobin, G.E. Biesecker and J.B. Greenhouse, JAMA, 275 (1996) 363.
[140] R.A. Goyer, Environ. Health Perspect., 100 (1993) 177.
[141] L. Moller and T.S. Kristensen, Am. J. Epidemiol., 136 (1992) 1091.
[142] R.A. Goyer and T.W. Clarkson, In: Toxicology, the Science of Poisons (ed. Klaassen, C.D.); McGraw-Hill, New York, 2001.
[143] W. Victery, H.A. Tyroler and R. Volpe, Environ. Health Perspect., 78 (1988) 139.
[144] Agency for Toxic Substances and Disease Registry (ATSDR), 1999

[145] L.J. Lawton and W.E. Donaldson, Biol. Trace Elem. Res., 28 (1991) 83.
[146] R. Sandhir, D. Julka and K.D. Gill, Pharmacol. Toxicol., 74 (1994) 66.
[147] S.J.S. Flora, J. Nutri. Env. Med., 12 (2002) 53.
[148] S.J. Stohs and D. Bagchi, Free. Rad. Biol. Med., 18 (1995) 321.
[149] N. Ercal, H. Gurer-Orhan and N. Aykin-Burns, Curr. Topics Med. Chem., 1 (2001) 529.
[150] B. Hultberg, A. Anderson and A. Isaksson, Toxicol., 156 (2001) 93
[151] H.P. Monterio, E.J.H. Bechara and D.S.P. Abdalla, Mol. Cell. Biochem., 103 (1991) 73.
[152] A. Hunderkova and E. Ginter E, Fd. Chem. Toxicol., 30 (1992) 1011.
[153] R.C. Patra, D. Swarup and S.K. Dwivedi, Toxicol., 162 (2001) 81.
[154] G. Saxena and S.J.S. Flora, J. Biochem. Mol. Toxicol., 18 (2004) 221.
[155] R.I. Bauer, V.D. Hasemann and T.J. Johansen, Biochem. Biophys. Res. Commun., 94 (1980) 1294.
[156] R. Sandhir and K.D. Gill, Biol. Trace Elem. Res., 48 (1995) 91.
[157] L. Magos and M. Webb, CRC Crit. Rev. Toxicol., 8 (1980)1.
[158] N. Aykin-Burns, A. Laegeler, G. Kellogg and N. Ercal, Arch. Env. Contam. Toxicol., 44 (2003) 417.
[159] S.R. Ribarov and L.C. Benov, Biochem. Biophys. Acta., 640 (1981) 721.
[160] Y.S. Jiun and L.T. Hsien, Arch. Environ. Health, 49 (1994) 256
[161] W.L. West, E.M. Knight, C.H. Edwards, M. Manning, B. Spurlock, H. James, A.A. Johnson, U.J. Oyemade, C.O. Jackson, and L.S. Westney, J. Nutri., 124 (1994) 981S.
[162] R.W. Carrell, C.C. Winterbourne and E.A. Rachmillewitz, Br J Haematol., 30 (1975) 259.
[163] S. Sassa, Enzyme, 28 (1982)133.
[164] H.P. Monteiro, D.S.P. Abdalla, A. Faljoni-Alario and E.J.H. Bechara, Biochem. Biophys. Acta, 881 (1986) 100.
[165] H.P. Monteiro, D.S.P. Abdalla, O. Augusto and E.J.H. Bechara, Arch. Biochem. Biophys., 271 (1989) 206.
[166] E.J.H. Bechara, M.H.G. Medeiros, H.P. Monteiro, M. Hermes-Lima, B. Pereira, M. Demasi, C.A. Costa, D.S.P. Abdalla, J. Onuki, C.M.A. Wendel and P.A Di Mascio, Quim. Nova, 16 (1996) 385.
[167] H. Gurer and N. Ercal, Free Radic. Biol. Med., 29 (2000) 927.
[168] P.P. Nair, H.S. Murthy and N.R. Grossman, Biochem. Biophys. Acta, 215 (1970) 112.
[169] T.M. Bray and CG Taylor, Can. J. Physiol. Pharmacol., 71 (1993) 746.
[170] T.I. Lidsky, and J.S. Schneider, Brain, 126 (2003) 5
[171] J.P. Bressler and G.W. Goldstein, Biochem. Pharmacol., 41 (1991) 479.
[172] M.W. Bradbury and R. Deane, Neurotoxicol., 14 (1993) 131
[173] L.E. Kerper and P.M. Hinkle, Toxicol. Appl. Pharmacol., 146 (1997) 127.
[174] L.E. Kerper and P.M. Hinkle, J. Biol. Chem., 272 (1997) 8346.
[175] R.P. Rubin, Pharmacol. Rev., 22 (1970) 389.
[176] M. Kern and G. Audesirk, Toxicol. Appl. Pharmacol., 134 (1995) 111.
[177] E.K. Silbergeld, Life Sci., 20 (1977) 309.
[178] S.M. Lasley, M.C. Green and M.E. Gildberg, Neurotoxicol., 20 (1999) 619.
[179] E.K. Silbergeld, FASEB J., 6 (1992) 3201
[180] L. He, A.T. Poblenz, C.J. Medrano and D.A. Fox, J. Biol. Chem., 275 (2000) 12175.
[181] D.A. Fox, M.L. Campbell and Y.S. Blocker, Neurotoxicol., 18 (1997) 645.
[182] M.F. Beal, E. Brouillet, B.G. Jenkins, R.J. Ferrante, N.W. Kowall and J.M. Miller, J. Neurosci., 13 (1993) 4181.
[183] G.S. Shukla, T. Hussain and S.V. Chandra, Life Sci., 41 (1987) 2215.
[184] W. Deng and R.D. Poretz, Toxicol. Appl. Pharmacol., 172 (2001) 98.

[185] J. Villeda-Hernandez, R. Barroso-Moguel, M. Méndez-Armenta, C. Nava-Ruíz, R. Huerta-Romero and C. Ríos, Brain Res. Bull., 55 (2001) 247.
[186] U. Rafalowska, L. Struzynska, B. Dabrowska-Bouta and A. Lenkiewicz, Acta Neurobiol. Exp., 56 (1996) 611.
[187] L. Struzynska, B. Dabrowska-Bouta and U. Rafalowska, Acta Neurobiol. Exp., 57 (1997) 275.
[188] M. Markowitz, I.C. Lemente and J. F. Rosen, Am. 1. Pub. Health, 89 (1999) 1088.
[189] N.C. Papanikolaou, E.G. Hatzidaki, S. Belivanis, G.N. Tzanakakis and A.M. Tsatsakis, Med. Sci. Monit., 11 (2005) 329.
[190] D.A. Cory-Slechta, B. Weiss and C. Cox, J. Pharmacol. Exp. Ther., 243 (1987) 804.
[191] F.P. Paloucek, Am. Pharm., NS33 (1993) 81.
[192] K.R. Mahaffey, Environ. Health Perspect., 103 (1995) 191.
[193] C.S. Fullmer, Environ. Res., 72 (1997) 45
[194] E.K. Silbergeld, J. Schwartz and K.R. Mahaffey, Environ. Res., 47 (1988) 79
[195] K. Kostial, D. Dekanic, S. Telisman, M. Blanuska, S. Duvanaic, D. Prpic-Majic and J. Pongracic, Biol. Trace Elem. Res., 28 (1991) 181.
[196] R.D. Baynes and T.H. Bothwell, Ann. Rev. Nutr., 10 (1990) 133.
[197] S. Piomelli, C. Seaman and S. Kapoor, Ann. NY Acad. Sci., 514 (1987) 278.
[198] P.L. Goering, Neurotoxicol., 14 (1993) 45
[199] S.J.S. Flora, J.R. Behari and S.K. Tandon, Toxicol. Lett., 13 (1982) 51.
[200] G.D. Miller, T.F. Massaro and E.J. Massaro, Neurotoxicol., 11 (1990) 99.
[201] S.J.S. Flora, S. Singh and S.K. Tandon, J. Inter. Med. Res., 17 (1989) 68
[202] S.J.S. Flora, D. Kumar and S. Das Gupta, Pharmacol. Toxicol., 68 (1991) 3.
[203] E.A. Border, A.C. Cantrell and T.A. Kilroes-Smith, Br. J. Ind. Med., 33 (1976) 85.
[204] T.M. Bray and W.J. Bettger, Free Rad. Bio. Med., 8 (1990) 281.
[205] S.J.S. Flora, S Singh and S.K. Tandon, Acta Pharmacol. Toxicol., 53 (1983) 28.
[206] A.I. Othman and M.A. El-Missiry, J. Biochem. Molecul. Toxicol., 12 (1998) 345.
[207] E. Tokarski and L. Reio, Acta Chem. Scand., B32 (1978) 375
[208] S.K. Tandon, S.J.S. Flora and S. Singh, Pharmacol. Toxicol., 60 (1987) 62
[209] S.J.S. Flora and S.K. Tandon, Ind. J. Physiol. Pharmacol., 31 (1987) 267
[210] S.J.S. Flora and S.K. Tandon, Trace Elem. Electr., 12 (1995) 131.
[211] A.B. Fischer, C. Hess, T. Neubauer and T. Eikmann, Analyst, 123 (1998) 55
[212] S.J.S. Flora, and S.K. Tandon, Acta Pharmacol. Toxicol., 58 (1986) 374.
[213] J.A. Simon and E.S. Hudges, JAMA, 281 (1999) 2289.
[214] M. Dhawan, D.N. Kachru and S.K. Tandon, Arch. Toxicol., 62 (1988) 301.
[215] R. Lauwerys, H. Roels and J.P. Buchet, J. Occup. Med., 25 (1983) 668
[216] A. Sohler, M. Kruesi and C.C, Pfeiffer, Ortholocul. Psychiatry, 6 (1977) 272.
[217] G.R. Bratton, J. Zmudzki, M.C. Bell and L.C. Warnock, Toxicol. Appl. Pharmacol., 59 (1981) 164.
[218] L.B. Sasser, G.G. Hall, G.R. Bratton and J. Zmudzki, J. Nutr., 114 (1984) 1816.
[219] S.J.S. Flora and R.P. Sharma, Biol. Trace Elem. Res., 10 (1986) 137).
[220] J.S. Kim, D.L. Hamilton and B.R. Blakely, Can. 1. Vet. Res., 56 (1992) 256.
[221] J.H. Cheong, D.O. Seo, J.R. Ryu, C. Shin, Y.T. Kim, H.C. Kim and K.H. Ko, Toxicol.,133 (1999) 105
[222] K.S. Ghazaly, Comp. Biochem. Physiol., 100c (1999) 417-421.
[223] Y. Ito, M. Sasaki, Y. Ohka, T. Shimohara, and I. Ishiguro, Jap. J. Clin. Chem., 10 (1986) 185.
[224] Y. Ito, Y. Niiya, M. Otani and S. Shima, Toxicol. Lett., 37 (1987) 221.
[225] M. Dhawan, S.J.S. Flora, S.K. Tandon, Biomed. Environ. Sci., 2 (1989) 335.
[226] G.R. Buetter, Arch. Biochem. Biophys., 300 (1993) 535.

[227] D.P. Jones, V.E. Kagan, S.D. Aust, D.J. Reed and S.T. Omaye, Fund. Appl. Toxicol., 26 (1995) 1.
[228] B. Halliwell, Nutr. Rev., 52 (1994) 253.
[229] P. Weber, A. Bendich and L.J. Machlin, Nutrition, 13 (1997) 450.
[230] V.N. Adonaylo and P.I. Oteizal, Toxicology, 135 (1999) 77
[231] Hermes-Lima, Free. Rad. Biol. Med., 19 (1995) 381.
[232] C. McGowan, Toxicol. Lett. 47 (1989) 87.
[233] B.M. Solliway, A. Schaffer, H. Pratt and S. Yannai, Pharmacol. Toxicol., 78 (1996) 18.
[234] R. Neal, P. Yang, J. Fiechtl, D. Yildiz and N. Ercal, Toxicol. Lett., 91 (1997) 169.
[235] R. Neal, K. Cooper, H. Gurer and N. Ercal, Toxicology, 130 (1998) 167
[236] H. Gurer, H. Ozgunes, S. Oztezcan and N Ercal, Free Radical Biol. Med., 29 (1999) 927.
[237] R.J. Hittable, Physiol. Rev., 72 (1992) 101.
[238] O. Anderson, Chem. Rev., 99 (1999) 2683.
[239] C.D. Klaassen: Goodman and Gilman's. The Pharmacological Basis of Therapeutics, Pergamon Press. USA (1990), pp 1592-1614.
[240] J.J. Chisholm, Jr., Am. J. Dis. Child., 141 (1987) 1256.
[241] S.J.S. Flora, R. Bhattacharya and R. Vijayaraghavan, Fund. Appl. Toxicol., 25 (1995) 233.
[242] C.F. Brownie, C. Brownie, D. Noden, L. Krook, M. Haluska and A.L. Aronson, Toxicol. Appl. Pharmacol., 82 (1986) 426.
[243] C.G. Roussaeux and L.G. MacNabb, J. Appl. Toxicol., 12 (1992) 35.
[244] M. Shannon, J. Graef and F.H. Lovejoy, J. Pediatric., 112 (1988) 799.
[245] Y.K. Soong, H.Y. Huang, C.C. Huang and N.S. Chu, J. Formos. Med. Assoc., 90 (1991) 693.
[246] C. Harvard and K. Kunze, Eur. Neurol., 34 (1994) 337.
[247] M. Shannon, A. Grace, and J.W. Graef, N. Engl. J. Med., 321 (1989) 979.
[248] D.A. Cory-Slechta, J. Pharmacol. Exp. Ther., 246 (1988) 84.
[249] N. Ercal, P. Treratphan, T.C. Hammond, R.H. Mathews, N.H. Grannemann and D.R. Spitz, Free Rad. Biol. Med., 21 (1996) 157.
[250] A.L. Miller, Alt. Med. Rev., 3 (1998) 199.
[251] J. Zhang, X. Wang, Z. Lu, N. Liu and B. Zhao, Free. Rad. Biol. Med., 37 (2004) 1037
[252] K. Hruby and A. Donner, Med. Toxicol., 2 (1989) 317.
[253] D.J. Thomas and J.J. Chisholm, Jr., Toxicologist, 6 (1996) 49 (abstract 191).
[254] K. Kalia and S.J.S. Flora, J. Occup. Hlth., 47 (2005) 1
[255] C.R. Angle. : Toxicology of Metals (Chang, L.W. Ed.) CRC Press, pp. 487-504 (1996)
[256] S.J.S. Flora and P. Kumar, Drug Invest. 5 (1993) 269.
[257] S.K. Tandon, S.J.S. Flora and S. Singh, Toxicol. Appl. Pharmacol., 79 (1985) 204.
[258] L.R. Cantilena Jr and C.D. Klaassen, Toxicol. Appl. Pharmacol., 58 (1981) 452.
[259] C.L. Bell and F.M. Grandjean, Arthritis Remed., 26 (1983) 801.
[260] S.J.S. Flora and S.K. Tandon, Trace Elem. Electr., 12 (1995) 131.
[261] S.J.S. Flora and S.K. Tandon, Toxicology, 64 (1990) 129.
[262] S.J.S. Flora, M. Dhawan and S.K. Tandon, Clin. Chem. Enzymol. Commun., 3, (1990) 97.
[263] S.J.S. Flora, Human Exp. Toxicol., 10 (1991) 331.
[264] S.J.S. Flora, R. Bhattacharya and S.R.S. Sachan, Pharmacol. Toxicol., 74 (1994) 330.
[265] S.J.S. Flora, S. Singh and S.K. Tandon, Life Sci., 38 (1986) 67.
[266] S.K. Tandon, S.J.S. Flora and S. Singh, Pharmacol. Toxicol., 60 (1987) 62
[267] J.S. Kim, B.R. Blakely and C.C. Rousseaux, J. Appl. Toxicol., 10 (1990) 93
[268] M. Dhawan, D.N. Kachru and S.K. Tandon, Arch. Toxicol., 62 (1988) 301

[269] S.K. Tandon, S. Singh and S.J.S. Flora, J. Trace Elem. Electro. Health Dis., 8 (1994) 75.
[270] M.M. Jones, Crit. Rev. Toxicol., 21 (1991) 209.
[271] A.D.C. Battle, S.R. Paredes, I. Fukuda, P .A. Kozicki and M.V. Rossetti, Biochem. Arch., 2 (1986) 293.
[272] S.R. Paredes, R.A. Kozicki, A.M.D. Battle and C. Del, Comp. Biochem. Physiol., 382 (1985) 751
[273] S.R. Paredes, De Suknat, A.A.J. Geralnik, A.M.D. Battle and H.A. Canti, Int. J. Biochem., 17 (1985) 625.
[274] E.M. Walker, A. Stone, L.B. Milligan, G.R. Gale, L.M. Atkins, A.B. Smith, M.M. Jones, P.K. Singh and M.A. Basinger, Toxicology, 76 (1992) 79.
[275] P.K. Singh, M.M. Jones, G.R. Gale, L.M. Atkins and A.B. Smith, Toxicol. Appl. Pharmacol., 97 (1989) 572.
[276] S.J.S. Flora, G.M. Kannan, B.P. Pant and D.K. Jaiswal, Arch. Toxicol., 76 (2002) 269-276.
[277] S.J.S. Flora, S. Bhadauria, S.C. Pant and R.K. Dhaked, Life Sci., 77 (2005) 2324.
[278] A. Mehta and S.J.S. Flora, Fd. Chem. Toxicol., 39 (2001) 1039.
[279] M. Blanusa, L. Prester, M. Piasek, K. Kostial, M.M. Jones and P.K. Singh, J Trace Elem. Exp. Med., 10 (1997) 173.
[280] M.A. Bosque, J.L. Domingo, J. Cobella, M.M. Jones and P.K. Singh, J. Toxicol. Environ. Health, 42 (1994) 443.
[281] M.W. Taubeneck, J.L. Domingo, J, M, Llobet and C.L. Keen, Toxicology, 72 (1992) 27.
[282] A. Mehta, G.M. Kannan, S.N. Dube, B.P. Pant, S.C. Pant and S.J.S. Flora, J. Appl Toxicol., 22 (2002) 359.
[283] S.J.S. Flora and A. Mehta, J. Appl. Toxicol., 23 (2003) 97.
[284] A. Mehta, S.C. Pant and S.J. S. Flora, Rep. Toxicol., 21 (2006) 94.
[285] M. Pande, A. Mehta, B.P. Pant and S.J.S. Flora, Environ. Toxicol. Pharmacol., 9 (2001) 173.
[286] K. Kostial, D. Dekanic, S. Telisman, M. Palunska, S. Duvanaic, D. Prpic-Majic and J. Pongracic, Biol. Trace Elem. Res., 28 (1991) 181.
[287] S.J.S. Flora, U. Gubrelay, G.M. Kannan and R. Mathur, J. Appl. Toxicol., 18 (1998) 357.
[288] J.B. Besunder, D.M. Super and R.L. Anderson, J. Pediatric., 130 (1997) 966.
[289] M.A. Peraza, F. Ayala-Fierro, D.S. Barber, E. Casarez and L.T. Raei, Environ. Hlth. Perspect., 106 (1990) 203.
[290] D.P. Jones, V.E. Kagan, S.D. Aust, D.J. Reed and S.T. Omaye, Fund. Appl. Toxicol., 26 (1995) 1.
[291] A.B. Fischer, C. Hass, T. Neubauer and T. Eikmann, Analyst, 123 (1998) 55.
[292] G. Saxena, U. Pathak and S.J.S. Flora, Toxicology, 214 (2005) 39.
[293] J.S. Kim, E.C. Crichlow, B.R. Blakley and C.G. Rousseaux, Vet. Human Toxicol., 32 (1994) 101.
[294] B.D. Kumar, M.M. Khan and K. Krishnaswamy, Ind. J. Pharmacol., 26 (1994) 277.
[295] B. Halliwell, M. Wasil and M. Grootveld, FEBS Lett., 213 (1987) 15.
[296] S.J.S. Flora, G.M. Kannan, B.P. Pant and D.K. Jaiswal, J.Environ. Sci. Health Part C, 38 (2003) 241.
[297] H.A. Waldron, Br. J. Ind. Med., 23 (1966) 83.
[298] R.J. Reiter, R.C.G. Carneiro and C.S. Oh, Horm. Metab. Res., 29 (1997) 343.
[299] L.R. Barlow-Walden, R.J. Reiter, M. Abe, M. Pablos, A. Menendez-Pelaes, L.D. Chen and B. Poeggeler, Neurochem. Int., 26 (1995) 15.

[300] S.J.S. Flora, M. Pande, G.M. Kannan and A. Mehta, Cell. Mol. Biol., 50 (2004) OL543-OL551
[301] L. Packer, E.H. Witt and H.J. Tritschler, Free Rad. Biol. Med., 19 (1995) 227.
[302] M. Pande and S.J.S. Flora, Toxicology, 177 (2002) 187
[303] S.J.S. Flora, A. Mehta, P.V.L. Rao, G.M. Kannan, A.S.B. Bhaskar, S.N. Dube, and B.P. Pant, Toxicology, 195 (2004) 127.
[304] S.R. Musk, M.N. Preobrazbenskaya, G.A. Belitsky, A.M. Korolev, T.A., Lytcheva, I.A. Khitr, and I.T. Johnson, Mutation Res., 323 (2004) 69
[305] S. Nocentini, M. Guggiari, D. Rouillard and S. Surgis, Phytochem. Photobiol., 73 (2001) 370.
[306] I.I. Koleva, T.A. Van Bleek, J.P. Linssen, A. De-Groot, and L.N. Evastatieva, Phytochem. Analysis, 12 (2001) 8.
[307] G. Saxena and S.J.S. Flora, J. Pharm. Pharmacol., (2006), In Press
[308] K. Nalini, A.R. Arroor, K.S. Karanth and A. Rao, Fitoterapie, LXIII (1992) 232.
[309] P. Nandi, G. Talukder and A. Sharma, Phyto. Res., 11 (1997) 273.
[310] S.S. Chaurasia, S. Panda and K. Anand, Pharmacol. Res., 41 (2000) 663.

Lead - J.S. Casas and J. Sordo (Editors)

Chapter 5

Analytical procedures for the lead determination in biological and environmental samples

P. Bermejo Barrera[a] and J.A. Cocho de Juan[b]

[a]Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry,University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

[b]Laboratory of Metabolic and Nutritional Disorders, Faculty of Medicine, University Hospital of Santiago de Compostela 15706 Santiago de Compostela, Spain

1. LEVELS OF LEAD AND THEIR IMPLICATIONS FOR ANALYTICAL TECHNIQUES

The average concentration of lead in the earth's crust is approximately 15 μg/g (ppm). In more common rocks the concentration is between 30 ppm in granite and black stats to 1 ppm in sediments, basalts and igneous rocks. Marine sediments are the final deposits of lead in the environment, their levels being between 15 and 30 ppm [1]. The level in water depends on the type of water; in water with little carbon dioxide and pH near to 7, the lead concentration is normally lower than 10 μg/L due to the combination of lead with sulfates, carbonates and hydroxides, which form insoluble compounds. Nevertheless, acidic waters can have higher amounts of lead. The level in river water is between 0.1 and 100 μg/L, in lakes 0.1-50μg/L, and in sea water 0.01-10μg/L [1].

The level of lead in blood plasma is about 0.08-0.48 μg/L, but in general the lead concentration in erythrocytes is 16 times higher than in plasma [2] (for this the lead is normally determined in whole blood). The concentration range in blood is between 40 and 290 μg/L. Levels differ in males and females: some authors [3] found 153 μg/L in adult males and 100 μg/L in adult females, while for children below 14 years figures of 94 μg/L (males) and 86 μg/L (females) were observed. In a study with 8635 subjects the correlation between lead levels, alcohol consumption and smoking was clearly supported by experimental data. A 25% reduction of lead concentration in blood was apparent when comparing

present values to those for the period 1979-1985 [4]. The gradual decrease in lead levels was ascribed only in part to a decrease of the metal in gasoline, while variations in wine consumption also had a major impact. For urine, the concentration range is 12-30 μg/L [4]. The variation observed in lead concentration in human milk could be due to differences of intake related to environmental conditions, although it cannot be excluded that at least a part of the variation may be caused by analytical errors and contamination with laboratory airborne dust, which is rich in this element. The values for Hungary, The Philippines and Sweden appear to be higher than those found in Guatemala, Nigeria and Zaire. The concentration range for milk is between 2 and 30 μg/L [4]. In healthy persons the concentration in scalp hair is 2-5 times greater than in bones, about 10-50 times greater than in blood and 100-500 times greater than that excreted in urine [5,6]. The concentration range is between 0.004 and 95 μg/g [4]. In the kidneys the concentration range is 0.11-0.41 μg/g and in the liver the levels are greater: 0.25-2.30 μg/g. The levels in lungs are very different depending on the subject. A reference value of 0.279 μg/g (with a 10^{th} to a 90^{th} percentile range of 0.142-0.380 μg/g) was given for subjects in an industrialized area of Italy [7]. Other authors confirm the susceptibility of the lead content in the lungs to the pollution in living or working environments. Such reference values vary from 10 ng/g for young people or inhabitants of non-polluted areas to 0.5 μg/g for heavily or long-term exposed subjects [8-12]. The concentration range obtained was 1.00-500 ng/g [4].

The level of lead present in environmental and biological samples varies between some ppm to a few ppb; the use of very sensitive analytical techniques is necessary. In these cases, different spectroscopic techniques such as molecular and atomic spectroscopies, mass spectroscopy and some electroanalytical techniques can be used for lead determination. Nevertheless, in some cases, the sensitivity is not sufficient and the use of a preconcentration method previous to the analytical determination will be necessary.

2. PREANALYTICAL STEPS: SAMPLING AND CONSERVATION

Due to the low level of lead present in environmental and biological samples, it is necessary to take some precautions in the sampling procedure and in the conservation of samples. All material used in sampling and to conserve the samples, i.e. plastic or glassware, must be soaked for at least 48 hours in 10% nitric acid and finally rinsed with ultrapure water before use.

The sample conservation depends on the type of sample. For water, sea water, river or tap water, due to the low levels (a few ppb), it is necessary to avoid contamination of the sample by the material used and losses of the lead by precipitation or by adsorption in the container wall. To avoid this, the addition of an acid, usually nitric acid, in a concentration of about 1% is recommended

and to store the water samples at 4°C if the analysis is to be carried out in a few days; but if the samples must be stored for a long period freezing at –18°C is recommended. To control possible contamination it is very important to use different types of blanks: transport blank, reagent blanks, etc.

For the analysis of soil, if the samples are dry they can be stored in a clean container at room temperature. For sediments, it is preferable to dry the sediment, normally by liofilization and store the sediment in a plastic bottle until use. The plamust be dried by liofilization and must be ground to obtain adequate particle size. The dry sample can be stored in a plastic bottle at room temperature until use.

Contamination problems for biological samples occur during sampling. The high potential for lead contamination in capillary blood samples during collection has been known for some time. Some workers have suggested using special steps to minimize lead contamination errors during sample collection. These include thoroughly scrubbing the hand and the finger to be punctured with soap and then alcohol; using dilute (0.3M) nitric acid; or using a barrier spray, e.g. silicone, to reduce errors due to lead contamination. The collection tubes, lancets and needles must be checked for lead contamination. The EDTA is the recommended anticoagulant. Samples must be stored a 4°C. For the analysis of lead in urine, if definitive data on urinary excretion of lead is required, a 24-hour urine collection yields the preferred specimen. The urine sample must be collected in a 1-2 L lead-free (acid leaching/washed) wide-mouthed voiding container for direct urine collection. The addition of preservatives to urine specimens, such as HCl or HNO_3, is not recommended as there is increased potential for contamination. The urine samples must be stored a 4°C, or at –18°C for long periods. For different tissues, the samples must be kept in lead-free plastic bottles and stored at –18°C until use.

3. SOME ANALYTICAL METHODS FOR LEAD DETERMINATION

3.1. UV-VIS absorption spectrometry

3.1.1. General aspects

UV-VIS spectra are obtained by measuring the intensity of monochromatic radiation across a range of wavelengths passing through a solution. Figure 1 represents a typical experiment where a light beam of intensity, Io, strikes a sample consisting of a quartz or glass cell containing a solution. After passing through the cell, the light beam has a reduced intensity, I, due to various factors: reflection losses at the cell window, absorption in the sample and by scattering of dispersed particles. Only the absorption losses are caused by the dissolved sample.

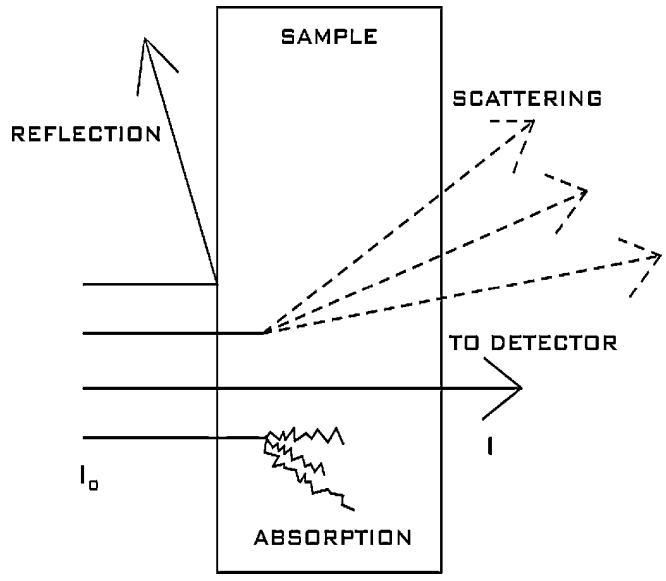


Figure 1. Losses of intensity of a light beam by reflection, scattering and absorption

The experiment is repeated using an identical cell containing only solvent to compensate for reflection and scattering losses. As a consequence of interaction between the photons and absorption particles, the beam is attenuated from Io to I. The transmittance, T, of the solution is then the fraction of incident radiation transmitted by the solution:

$$T = I / Io$$

The absorbance, A, of a solution is defined by the equation:

$$A = - \log T = \log Io / I$$

The absorbance of a solution increases as the attenuation of the beam becomes greater. The absorbance of a sample is proportional to the total amount of material that absorbs the incident light. It is possible to show that:

$$A = a\ b\ c \qquad (1)$$

Where a is a constant that is a property of the material itself as well as the wavelength of the measurement, b is the length of the path through which the light travels in the sample, and c is the concentration of the material that absorbs

the light. The equation (1) describes the **Beer-Lambert Law**, which is the principal law in Spectrometry. When the concentration c is in mol L^{-1}, b is in cm, then the constant a is expressed in L $mol^{-1}cm^{-1}$ and in this case, it is given the symbol ε and is called the molar extinction coefficient or molar absorptivity. This law is also applied to a solution containing more than one absorbing species. If there is no interaction between the various species, the total absorbance is given by:

$$A_{total} = A_1 + A_2 + \ldots\ldots + A_n = \varepsilon_1 b c_1 + \varepsilon_2 b c_2 + \ldots\ldots + \varepsilon_n b c_n$$

where the subscript refers to absorbing components 1,2,.....,n.
The Beer-Lambert Law presents some limitations of application [13].

3.1.1.1. Real limitations

The Beer-Lambert Law is valid for describing the absorption behavior of a dilute solution (usually < 0.01 M). At high concentrations, the distance between the absorbing species diminishes and it is possible that one species can affect the charge distribution of its neighbors and thus can alter the species' ability to absorb. The extent of this interaction depends on the concentration; this effect causes deviations from the linear relationship between absorbance and concentration. On the other hand, when the solution contains high concentrations of electrolytes, a similar effect due to electrostatic interactions is possible.

3.1.1.2. Apparent chemical deviations

When an analyte dissociates, associates, or reacts with a solvent to produce a product with different absorption spectra from the analyte, an apparent deviation of the Beer-Lambert Law occurs. The most common example of this behavior is produced in the acid/base indicator which depends on the pH, the system can present two different absorbing species, HIn (color 1) and In^- (color 2)

$$HIn \leftrightarrow H^+ + In^-$$
color 1 color 2

3.1.1.3. Instrumental deviations with polychromatic radiation

The Beer-Lambert Law is observed only in monochromatic radiation, but unfortunately, the use of radiation of a single wavelength is difficult, because it is difficult to isolate a single wavelength from a continuous source, normally an approximately symmetrical band of wavelengths around the desired one is obtained. Nevertheless, it is an experimental fact that these types of deviation are not appreciable, provided the radiation used does not encompass a spectral

region in which the absorber exhibits large changes in absorption as a function of wavelength. It is possible to show experimentally that the absorbance measurements at the maximum of narrow peaks, the deviations of the Beer-Lambert Law, are not significant if the effective bandwidth of the monochromator is less than 1/10 the half width of the absorption peak at half height.

3.1.1.4. Instrumental deviations due to stray radiation

The radiation can be contaminated with small amounts of scattered or stray radiation. Stray radiation usually has a different wavelength from the principal radiation and moreover may not have passed through the sample. It is possible to correct the absorbance value with the effects of the stray radiation, but in modern spectrophotometers this effect is not very important.

3.1.2. Absorbing species

The absorption of radiation UV-VIS by a molecular species, M, can be considered a two-step process; in the first step electronic excitation is produced obtaining an electronically excited species, M*, with a very brief lifetime (10^{-8}–10^{-9} s). The second step is normally a relaxation process that involves the conversion of the excitation energy to heat. The relaxation process produces the decomposition of M* to form a new species or may involve fluorescent or phosphorescent re-emission of radiation.

$M + h\nu \rightarrow M^*$
$M^* \rightarrow M + \text{heat}$

The absorption of UV-VIS radiation normally produces excitation of bonding electrons; for this the wavelengths of absorption peaks can be correlated with the types of bond in the absorbing species. In this case, molecular absorption spectrometry is adequate for identifying functional groups in a molecule. Nevertheless, the most important application of UV-VIS absorption spectrometry is quantitative determination of compounds containing absorbing groups [13].

The absorbing species containing π, σ and n electrons include organic molecules, ions and inorganic anions. The energies for the various types of molecular orbitals are very different. Normally, the energy level of a nonbonding electron lies between the bonding and the antibonding π and σ orbitals.

One electron in a bonding σ orbital is excited to the antibonding orbital by absorption of radiation. The amount of energy required to induce this transition is large and corresponds to radiant frequencies in the vacuum ultraviolet region. This region presents experimental difficulties due to the components of the atmosphere absorbed in this region.

The $n \rightarrow \sigma^*$ transitions are produced in saturated compounds containing atoms with unshared electron pairs. These transitions require less energy than $\sigma \rightarrow \sigma^*$ transition and are normally produced in the region between 150 and 250 nm. The $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions are the most important transitions for the absorption spectroscopy for organic compounds, because these transitions are in a convenient spectral region between 200 and 700 nm. For both transitions the presence of an unsaturated functional group is necessary to provide the π orbital. These groups are usually called chromophores. The transitions $\pi \rightarrow \pi^*$ show bigger molar absorptivities than the $n \rightarrow \pi^*$ transitions. On the other hand, another difference between both transitions is the effect exerted by the solvent on the wavelength of the peaks. The peaks of the transitions $n \rightarrow \pi^*$ are shifted to a shorter wavelength with increasing the solvent polarity, a blue shift, whereas for the transitions $\pi \rightarrow \pi^*$ the inverse effect is more common, a red shift, produced when the solvent polarity is increased.

In Table 1 we can see the most common organic chromophores with their absorption maxima. This data can be used as a guide for the identification of functional groups but with many limitations because the positions of maxima are affected by solvent and structural details of the molecule containing the chromophore. Peaks are normally broad due to vibrational effects, the precise determination of a maximum position being difficult.

Table 1
Absorption properties of some chromophores

Chromophore	Example	Solvent	λ_{max} (nm)	ε_{max}	Type of transition
Alkene	$C_6H_3CH=CH_2$	n-heptane	177	13000	$\pi \rightarrow \pi^*$
Carbonyl	CH_3COCH_3	n-hexane	186, 280	1000, 16	$n \rightarrow \pi^*, n \rightarrow \sigma^*$
Carboxil	CH_3COOH	Ethanol	204	41	$n \rightarrow \pi^*$
Amido	CH_3CONH_2	Water	214	60	$n \rightarrow \pi^*$
Nitro	CH_3NO_2	Isooctane	280	22	$n \rightarrow \pi^*$

An important aspect of chromophores is the effect of conjugation of chromophores that is produced when the groups are near each other. The effect of this delocalization is to lower the energy level of the π^* orbital and the absorption maxima is shifted to a longer wavelength. For example, 1,3-butadiene has a strong absorption band that is displaced to a longer wavelength in comparison with the corresponding peak for an unconjugated diene. Moreover, the conjugation between the double bonded oxygen of aldehydes, ketones and carboxilic acids and an olefinic double bond gives rise to similar behavior.

The absorption spectra of aromatic hydrocarbons are characterized by three bands due to $\pi \rightarrow \pi^*$ transitions. For benzene there is a strong absorption peak at 184 nm, a weaker band, E_2 band, at 204 nm and a weaker peak, B band, at 256 nm. All these bands are strongly affected by ring substitution; for example the E_2 band is displaced to 211 nm for phenol and to 230 nm for aniline. These groups –OH and $-NH_2$ are called auxochrome groups. An auxochrome group is a functional group that does not itself absorb but has the effect of shifting chromophore peaks to longer wavelengths as well as increasing their intensities. These groups have at least one pair of n electrons that interact with the π electrons of the rings, and as a consequence of this interaction a stabilizing effect on the π^* state is produced, lowering its energy and producing a red shift.

Some inorganic anions show ultraviolet absorption peaks due to $n \rightarrow \pi^*$ transitions, some examples are nitrate (313 nm), carbonate (217 nm), and nitrite (360 and 280 nm).

The lanthanide and actinide ions present absorption spectra in the ultraviolet and visible regions with narrow, well-defined and characteristic absorption peaks. These peaks are related to the energy levels of 4f and 5f electrons and are not usually affected by the ligand associated with the metal ion. Due to the inner character of these orbitals, these absorption peaks are relatively unaffected by the nature of the solvent.

The elements of the first and second transitions-metal series present ions and complexes with absorption spectrum in the UV and VIS range. In general, the absorption bands are broad and are very influenced by the environment. The most typical example is the pale blue color of the ion copper (II) in water and the dark blue of the tetraamine copper(II) complex. The electronic configuration of metals of the transition series is characterized by partially occupied d orbitals, and the absorption bands are due to the electronic transitions among the various energy levels of these orbitals. To explain the color of the transition metal-ions and their complexes, there are two theories: the crystal-field theory and the molecular-orbital theory. The first is simple and is adequate for a qualitative description, whereas the second is more complex and gives a quantitative description. When a metal forms a complex with a ligand in solution the splitting of the d-orbital energies occurs. This is due to the differential forces of electrostatic repulsion between the electron pair of the donor and the electrons in the d orbitals of the central metal ion. The splitting of the d orbitals can be different depending on the geometry of the complex; the three most common cases are the octahedral, tetrahedral and square planar. In all cases, the d orbitals of the metal in the presence of a ligand field are splitting in two or more orbital groups with different energy and are possible transitions between orbitals of low energy and orbitals of high energy. In general, these transitions correspond to the VIS spectra.

The complexes with charge transfer absorption are very important in analytical chemistry because they present very high molar absorptivities (greater than 10000) being adequate to determine absorbing species. In a charge-transfer complex it is necessary that one of its components has electron-donor properties (Lewis base) and the other component has electron-acceptor properties (Lewis acid). The absorption of radiation is produced when an electron is transferred from the donor to the acceptor. One typical example is the iron(III)-thiocyanate complex. In this complex the absorption is produced when an electron is transferred from the thiocyanate ion to an orbital associated with the iron(III). The product is an excited species of iron(II) and the neutral thiocyanate radical. The organic compounds can also form charge transfer complexes, for example the 1:1 complex between quinone and hydroquinone.

3.1.3. Spectrometers: general concepts

The instrument used to study the absorption of radiation in the UV-VIS region is made up of five components: 1) a stable source of radiant energy; 2) a wavelength selector to isolate a region of the spectrum; 3) a sample container; 4) a radiation detector, to convert the radiant energy to a measurable electrical signal; and 5) a signal processing and readout unit. Figure 2 shows the way these components are configured.

The most common source used in UV-VIS Spectroscopy is a tungsten filament lamp that provides a distribution of wavelengths from 320 to 2500 nm. The tungsten/halogen lamp, which contains a small amount of iodine within the quartz envelope that houses the filament, gives higher intensities and extends the ranges into the UV region up to 240 nm. On the other hand, the lifetime of a tungsten/halogen lamp is more than double that of an ordinary tungsten lamp because the problem of the sublimation of tungsten from the filament is avoided. Deuterium or hydrogen lamps are used to provide continuous radiation in the UV region.

To isolate the desired wavelength band a filter or a monochromator must be used. With the use of the filters we can select a specific band of wavelength to perform the measurements, but with the monochromator we can vary the wavelength to obtain the absorption spectra. Filters used for absorption measurements are usually interference filters based on optical interferences, and transmit radiation between 5 and 20 nm. These filters present some advantages such as simplicity, ruggedness and low cost, but are limited in application as they can only isolate one band of wavelengths.

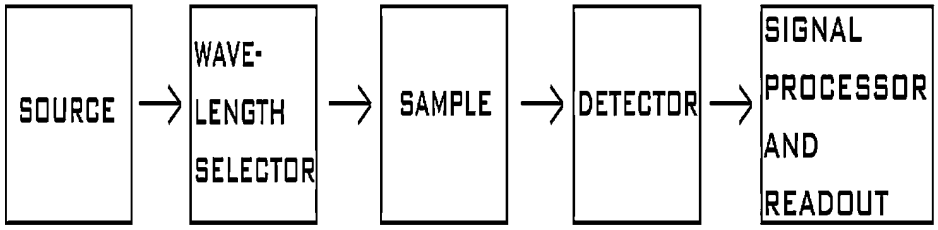


Figure 2. Components of a spectrometer

Monochromators are designed to perform spectral scanning. A monochromator contains: 1) an entrance slit; 2) a collimating mirror or lens that produces a parallel beam of radiation; 3) a dispersion element (a prism or a grating that disperses the radiation into its component wavelengths); 4) a focusing element that focuses the image of the slit on a focal plane; and 5) an exit slit in the focal plane that isolates the desired spectral band. Today, monochromators generally employ a diffraction grating; by rotating the grating, different wavelengths can be made to pass through an exit slit. The output wavelength of a monochromator is thus continuously variable over a spectral range. The spectral bandpass of a monochromator can be less than 1 nm.

The sample container, cell or cuvette, must be transparent in the spectral region of interest. Thus in the VIS region the cell can be plastic or silicate glass, but for the UV region it must be made of quartz or fused silica. The most typical cell has windows that are perpendicular to the direction of the radiation beam to minimize reflection losses. The most common length is 1 cm although it is possible to use longer cells. An important aspect is to take precautions in the use of the cells. Fingerprints, grease or other residues on the walls can alter the transmission properties of the cell. For this reason cleaning is an important operation in order to obtain a perfectly clean cell.

The detectors used in UV-VIS spectroscopy are photon detectors and can be phototubes, photomultiplier tubes, silicon photodiodes and photodiode arrays. The phototube or a photomultiplier tube is based on the photoelectric effect. In the phototube a photoemissive material emits electrons when it is irradiated with light. The emitted photoelectrons are attracted to an anode and a photocurrent is produced. This current is amplified and measured. The number of photoelectrons ejected from the cathode is directly proportional to the radiant power of the beam striking the surface. The photomultiplier tube is similar to the phototube but is more sensitive because a series of additional electrodes called dynodes are enclosed in the system and an electron amplification is produced.

Photodiodes are semiconductor pn junction devices that respond to incident light by forming electron-hole pairs. Silicon photodiode detectors respond very quickly, in nanoseconds. Although sensitivity is less than the

photomultiplier tubes, an advantage is the possibility of its fabrication in arrays of 1000 or more detectors and this allows simultaneous measurement at different wavelengths.

3.1.4. Instruments

To measure absorbed radiation it is possible to use two types of instruments: photometers and spectrophotometers. A photometer employs a filter for wavelength selection in conjunction with a suitable detector. It has the advantage of simplicity, ruggedness and low cost, and is normally used to study the VIS region of the spectra. On the other hand, photometers are normally used as detectors in other analytical techniques such as chromatography, electrophoresis, inmunoassays or continuous flow analysis. Spectrophotometers offer the advantage that the wavelength can be varied continuously, making it possible to obtain absorption spectra. They usually cover the UV-VIS region and sometimes the near-infrared region.

Both photometers and spectrophotometers can be obtained in single and double-beam varieties. Figure 3 shows a single-beam spectrophotometer which is designed for the visible region of the spectrum. This type of instrument is adequate for quantitative absorption measurement at a single wavelength. The most important advantages are the simplicity of instrumentation, low cost and ease of maintenance.

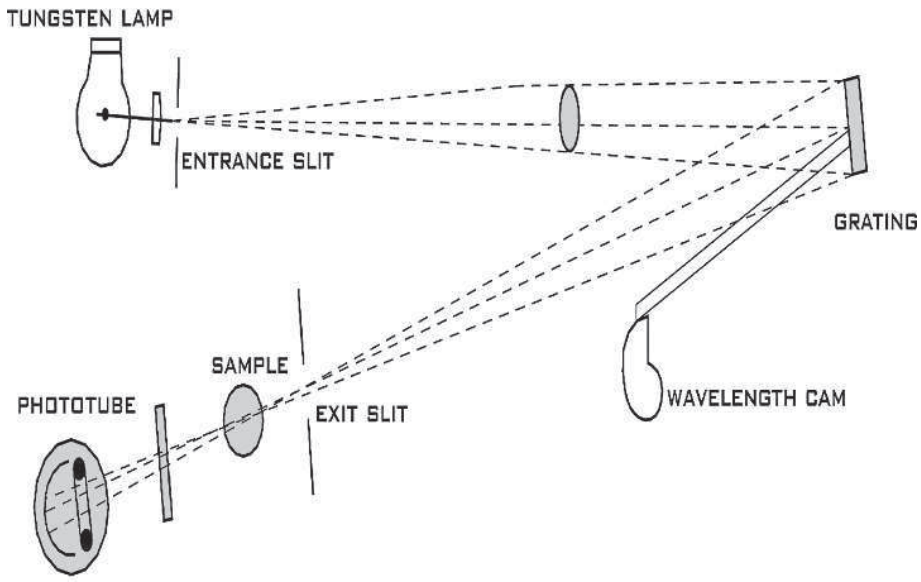


Figure 3. A single-beam spectrophotometer for VIS region

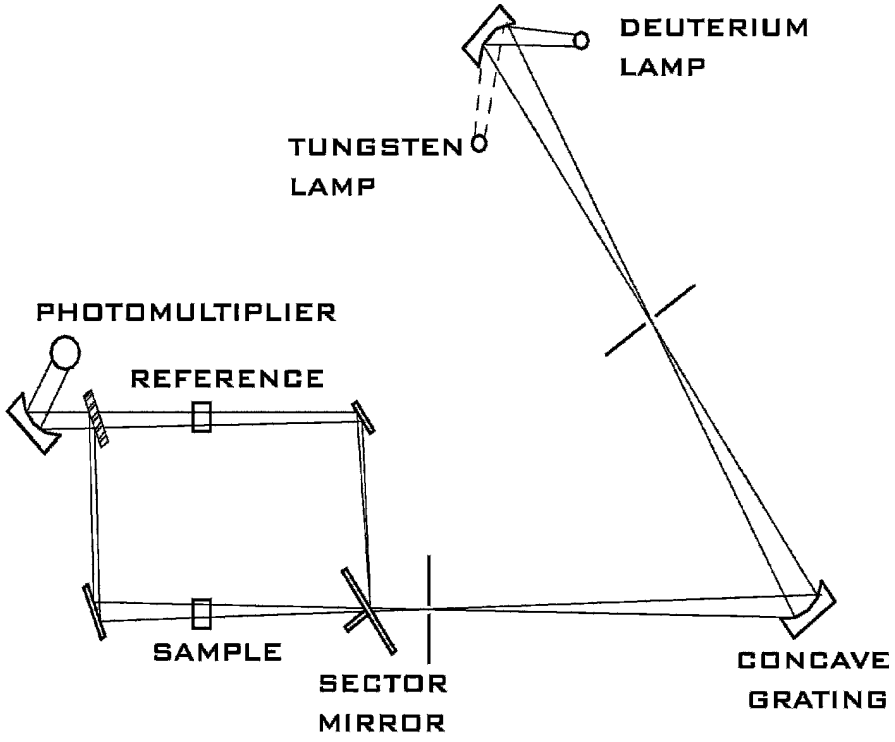


Figure 4. A double beam spectrophotometer for UV-VIS region

Figure 4 shows a double-beam design for the visible and ultraviolet regions. The radiation from the tungsten or deuterium lamp is dispersed by a concave grating, which also focuses the beam passing through the sample and the other passing through the reference cell. After that the beams reach a grid mirror and then the detector.

With the use of the silicon diode arrays as detectors it is possible to design multichannel instruments. Figure 5 shows an optical diagram of a multichannel UV-VIS spectrophotometer. This instrument is characterized by the fact that it presents few optical components, which means the radiation is much higher than in traditional spectrophotometers. After passing the radiation through the cell, it is focused on an entrance slit and then passes onto the surface of a reflection grating. The detector is a diode array and allows simultaneous measurement at different wavelengths. A single scan from 200-820 nm requires only about 0.1 s.

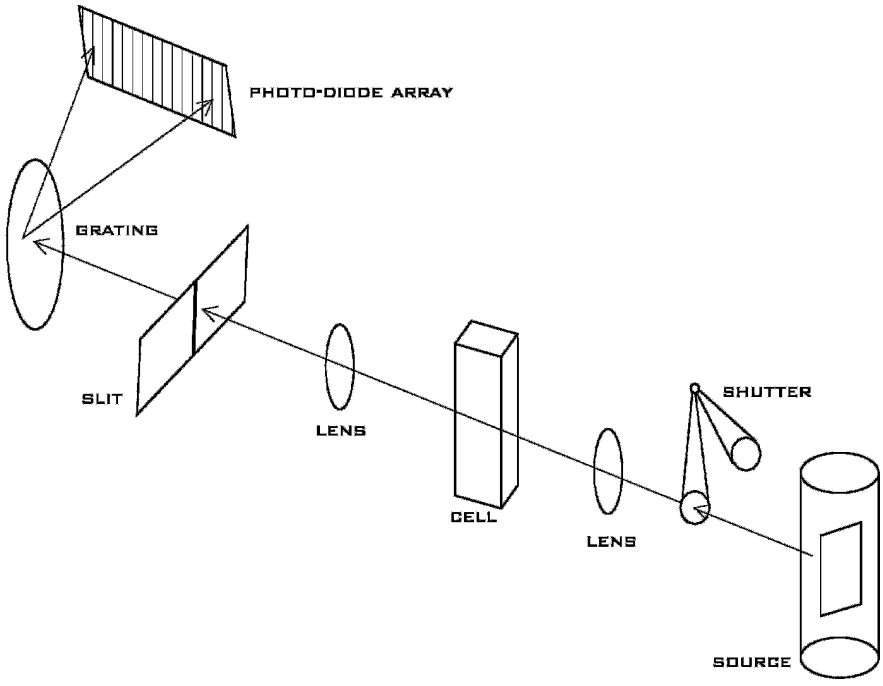


Figure 5. Diagram of a multichannel spectrophotometer based on a grating spectrograph with a photodiode array detector

3.1.5. Spectrophotometric determination of lead

The most important method used to perform the spectrophotometric determination of lead is the dithizone method. The dithizone (diphenylthiocarbazone) H_2Dz presents a high capacity to form metallic complexes acting as a monobasic acid [14]. For lead the complex has the formula $Pb(HDz)_2$ [15]. This complex is extracted in chloroform or in carbon tetrachloride, and has a pink-red color with absorption maxima of 510-520 nm with a molar absorptivity of $6.3.10^4$ [16]. The Lambert-Beer Law is linear up to 26 mg/L at 510 nm and up to 0.4 mg/L at 520 nm for a cuvette of 10 cm. The pH for a quantitative extraction must be between 8.5 and 11.5. To avoid the precipitation of metallic oxides, the addition of tartrate or citrate and cianure is necessary to avoid iron interference [17].

The dithizone method has been applied to lead determination in different matrices such as water, foods, blood, urine, etc. For solid samples a previous step of organic matter decomposition is necessary. Wet digestion procedures with the mixture $HNO_3/HClO_4/H_2SO_4$ or dry procedures such as calcination are

used for this. The dithizone method, although a laborious procedure, allows us to obtain precise, accurate and interference-free results. The automation of the complex formation and extraction steps, avoiding the need for any particular ability on the part of the operator, made the use of the method in routine analysis possible and resulted in its widespread use for many years. However, today, except in cases where instrumental techniques are not available, it is seldom used due to its poor sensitivity and the possibility of interference of metals such as thallium and cadmium that are co-extracted with lead.

In the literature it is possible to see many modifications of the dithizone method for lead determination, e.g., a first reaction of lead with sodium diethyldithiocarbamate, then extraction with a mixture 1:1 of pentanol-toluene. After the organic layer is treated with HCl and the lead complex passes through the aqueous phase, dithizone is added and the dithizone complex is extracted in CCl_4 [18-21].

Other reagents proposed for the colorimetric determination of lead were: azoderivative of the antranilic acid [22], 8-hydroxiquinoleine [23], blue of methyltimol [24], 8-hidroxy-7-α(2-metoxicarbonilamylic)benzyl) quinoleina [25] and xylenol orange [26].

3.2. Atomic absorption spectrometry (AAS)

3.2.1. General aspects

AAS is based on the absorption of radiation by free atoms, principally atoms in their ground state. Using a wavelength for an element that corresponds to an optical transition between atoms in the ground state and atoms in an excited level, an absorption of radiation is produced and the value of this absorption is related to the concentrations of atoms in the ground state, and therefore to the element concentration.

Optical transitions used for AAS measurements are generally between the ground state and the first excited state. Using Boltzman's law, it is possible to show that at the temperatures normally used to obtain free atoms, most atoms are in the ground state, and since the absorption of radiation is proportional to the number of atoms in the ground state, this explains why AAS is an efficient method.

An atomic absorption spectrometer consists of a primary radiation source which produces the radiation to be absorbed, a source of free atoms, usually named atomizer, an optical dispersive system, a detector and electronics for data acquisition, processing and editing. Figure 6 shows a schematic diagram of an atomic absorption spectrometer. The most typical components of the AAS

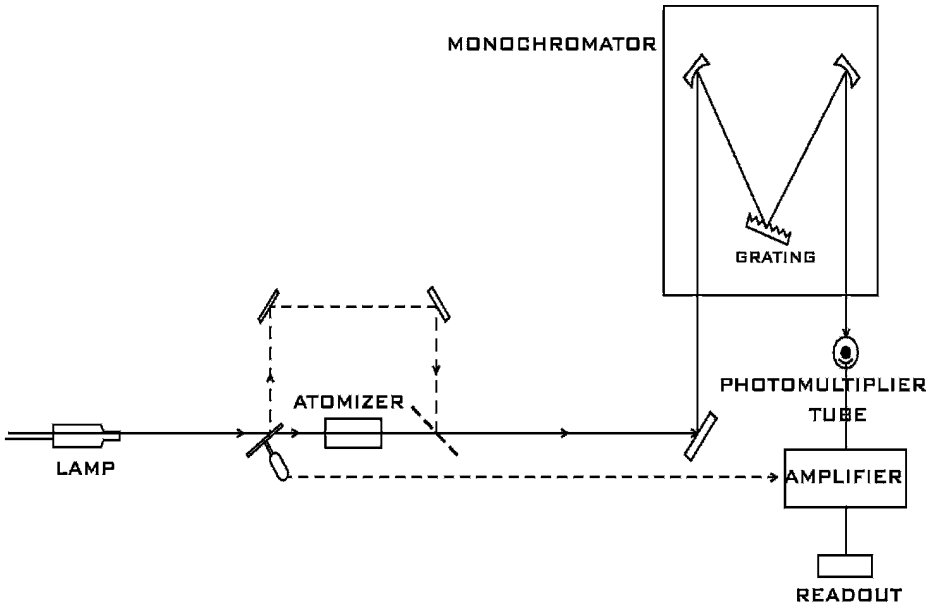


Figure 6. Schematic diagram of an atomic absorption spectrometer

spectrometer are the radiation source and the system used to obtain the free atoms in the ground state, the atomizer.

The primary radiation sources can be: hollow-cathode lamps (HCL) or electrodeless discharge lamps (EDL). The hollow cathode lamp consists of a hollow cathode made of a metal whose spectrum is to be produced. In some cases the cathode can be made by several metals obtaining a multi-elemental HCL. The cathode and the anode are in a glass cylinder with a silica window and filled with an inert gas such as argon or neon at a pressure of 1-5 torr. When a potential of 300 V is applied across the electrodes with a current of about 5-20 mA, the ionization of the inert gas occurs. If the potential applied is sufficient, the ions acquire enough kinetic energy to liberate some metal atoms from the cathode surface producing an atomic cloud; this process is known as sputtering of the atoms. The sputtered metal atoms can be excited by interaction with more argon ions, and thus emits their characteristic radiation when they return to the ground state. The cathode is regenerated because its cylindrical configuration enhances the probability that redeposition will occur at the cathode rather than on the glass walls.

For volatile elements, the energy emitted by the HCL must be relatively low. In these cases EDL lamps are used. These lamps provide radiant intensities one or two orders of magnitude greater than the HCL. An EDL consists of a

sealed silica tube containing the element or salt of interest with argon gas. The lamp has no electrode, the energy is supplied by an intense field of radio-frequency or microwave radiation, which produces the argon ionization to give ions with the sufficient energy to excite the atoms of the metal whose spectrum is desired. EDLs are generally used for elements such as: As, Cd, Hg, Pb, Sb, Se and Sn.

Boosted discharge HCLs are another intense primary radiation source also used for volatile elements. These lamps are similar to the HCL but add a secondary boost discharge to increase the excitation of atoms sputtered by the cathode and to minimize self-absorption. The intensities of these lamps are 5-15 times higher than a standard HCL.

In an atomic absorption spectrometer it is necessary to eliminate the emission of radiation by the flame in order to discriminate between the light emitted by the primary radiation source and that emitted by the atomizer. For this it is necessary to perform source modulation. This can be performed mechanically by means of a rotating chopper or electronically by modulating the HCL current using a pulsed power supply, the latter being the most common system. The atomizer is used to convert the sample to free atoms in the ground state. It must be located in the light path between the primary radiation source and the dispersive system. An ideal atomizer must produce the complete sample atomization. Two types of atomizer are commonly used for lead determination: the flame and the electrothermal atomizer.

3.2.2. Flame atomization (FAAS)

In flame atomization the liquid sample is sprayed into a flame by means of a nebulizer which converts the sample solution into an aerosol, with droplets of different sizes. The most common type of nebulizer is the concentric tube type. In this nebulizer the liquid sample is aspirated through a capillary tube by a high-pressure stream of gas flowing around the tip of the tube. The gas at high velocity breaks the liquid sample up into fine droplets of various sizes which are transported to the flame. Other types of nebulizer are the cross-flow nebulizer in which the high-pressure gas flows across a capillary tip at right angles. The aerosol formed in the nebulizer is mixed with the fuel and the oxidant gases in a mixing chamber. This chamber contains a series of baffles or an impact ball that only allows the smallest droplets to pass to the flame. The majority of the sample (bigger droplets) is collected in the bottom of the mixing chamber, where they are drained into a waste container. Thus, normally only a 10% of the sample can reach the flame. This is an important limitation of this atomization system which has a low efficiency. The aerosol, oxidant and fuel are then burned in a slotted burner, which provides a flame that is usually 5 or 10 cm in length. The laminar flow burners used in the flame atomizers provide a quiet

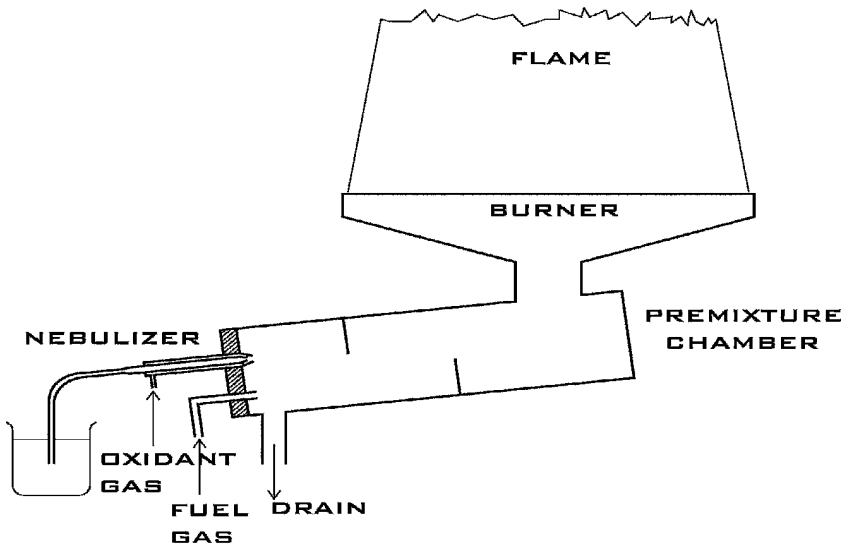


Figure 7. Typical laminar flow burner

flame and a long path length, this is important to increase the sensitivity and to improve the reproducibility. Figure 7 shows a scheme of a typical laminar flow burner.

The most common flames used in AAS are the air-acetylene flame and the acetylene-nitrous oxide flame. With the former, it is possible to obtain temperatures between 2100 and 2400°C and this flame is commonly used for the determination of elements whose oxides are not refractory such as Ca, Cr, Cu, Co, Fe, Ni, Mg, Sr, etc., whereas with the acetylene-nitrous oxide flame the temperatures can reach 2600-2800°C and it is used for elements such as Al, Si, Ta, Ti, V, Zr, etc.

The flow rate of the fuel-oxidant mixture is an important variable to control, and depends upon the kind of fuel and oxidant used. If the flow rate does not exceed the burning velocity, the flame moves back into the burner producing flashback.

Another important aspect is the zone of the flame used to observe the atomization process. In a typical flame there are three different zones. First is the primary combustion zone, with blue color due to band spectra of C_2, CH and other radicals. In this zone thermal equilibrium is not reached, so it is not used for flame spectrometry. The second zone is the interconal area, which is rich in

free atoms and is the zone used in the majority of applications. The last zone is the outer cone; it is the most external zone and is a secondary reaction zone in which the products of the inner cone are converted to stable molecular oxides. The temperature of the flame is different in the three zones, the interconal region having the highest temperature.

The behavior of the different elements according to the height of the flame can be very different. For example, magnesium presents a maximum absorbance in the middle of the flame. First the absorbance increases with the height of the flame because the number of magnesium atom produced increase, but when the outer zone is reach the oxidation of magnesium is produced and this produces a decrease in the absorbance because the oxide does not absorb at the wavelength. In contrast, the behavior of silver is very different: the absorbance always increases with the height because the silver is not oxidized. On the other hand, chromium, which forms very stable oxides, shows a continuous decrease of absorbance with the height of the flame. For this reason, a different portion of the flame must be selected for each element.

In Figure 8 we can see a scheme of the different processes that occur in a flame atomizer. The liquid sample is first transformed into an aerosol that is mixed with the fuel and the oxidant and is heated in the flame where the solvent is eliminated. After that, the sample passes through different transformations until it becomes molecules and atoms in the ground state, and these atoms absorb the radiation from the corresponding lamp.

There are several limitations associated with the flame. The first type is related to the structure of the flame and may be avoided by modifying the flame. The disadvantages are:

1) The conventional nebulizer and flame systems need relatively large volumes of solution. The efficiency of the nebulizer process is poor, only about 10% of solution uptake being delivered to the flame. Other nebulization systems can be used to improve efficiency, such us ultrasonic nebulization, heated spray chambers or hot gases.

2) Atom concentrations in flames are limited by the dilution effects of the relatively high flow-rate of gas used to support the flame, and by the flame gas expansion which occurs on combustion. The lifetime of atoms in the flame is very short: about 10^{-4}s.

3) Due to the short transit time in the flame, the cell solutions with high sample matrix or analyte concentrations may undergo incomplete solute vaporization and gaseous dissociation. Some elements can produce compound formation whereas some can be strongly ionized in flames, for example elements such as Na, K, Rb, Cs.

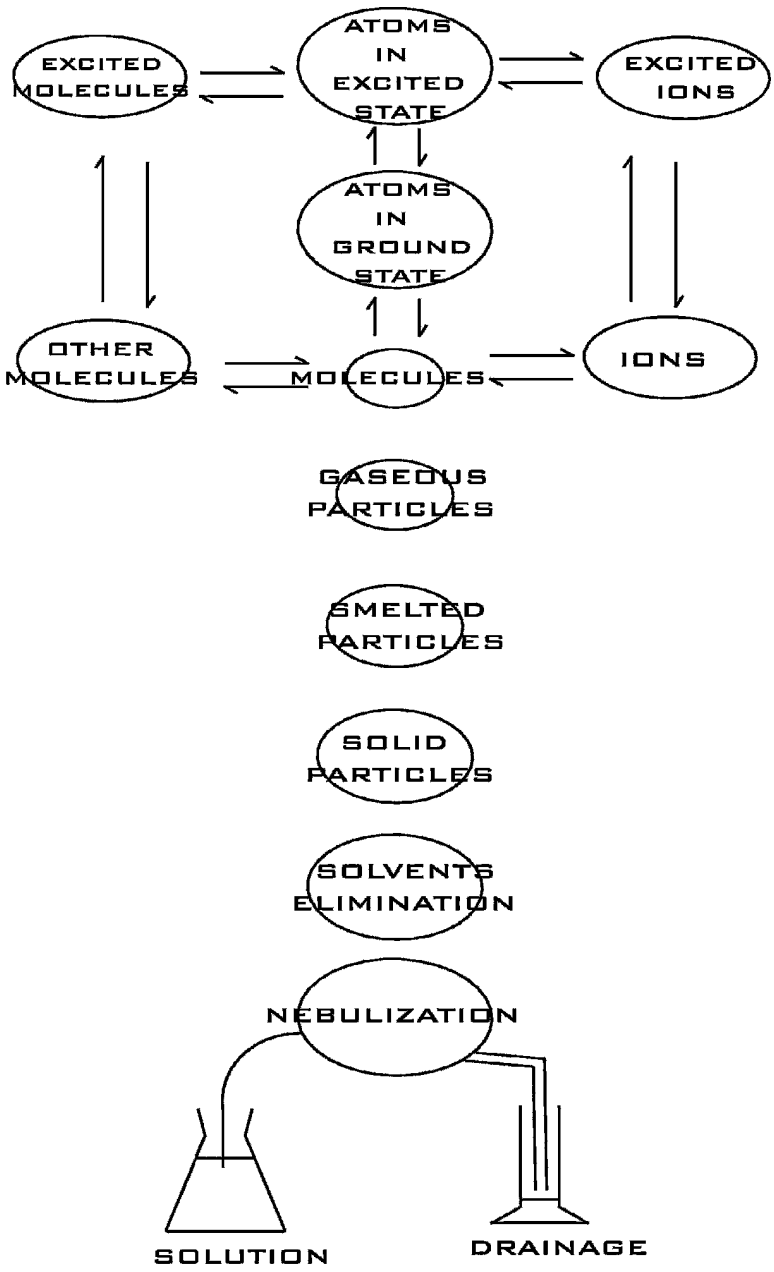


Figure 8. Process that happen in a flame atomizer

4) With the flame it is necessary to use liquid samples, but in certain situations the analysis of solid samples may be preferred. It is difficult to nebulize viscous samples and some organic solvents may extinguish the flame.

To overcome some of these problems, some modifications to the conventional flame atomizer have been proposed. One is the discrete sample nebulization of the injection technique. In this system a cup made of Teflon is attached to the nebulizer tube and the sample is pipetted into the cup using a micro-pipette; all the sample is consumed and a peak signal is observed [27]. This procedure permits the use of smaller samples (25-500 μL) with high salt concentrations over long periods of time without negatively influencing the function of the nebulizer and burner. This technique was investigated by different authors [28-33] and applied to microanalysis in particular. In general, absolute sensitivities and limits of detection are improved [28-30]; also, when small volumes are sprayed, higher efficiency of nebulization is observed. Another technique is the sampling boat [34]. This system uses a tantalum sampling boat from which the sample was evaporated as it was pushed into the flame. The technique gave a notable improvement in sensitivity but it is only applicable to the more-easily atomized elements and reproducibility is not very good. A modification of the sampling boat is the Delves sampling cup [35]. In this system the tantalum boat is replaced with a smaller (10 mm outer diameter, 5 mm deep, 0.15 mm metal foil) and more-easily positioned nickel microcrucible (or stainless-steel crucible). This crucible is mounted onto a device that enables it to be pushed close to the flame to allow charring and then into the flame to allow atomization. A nickel absorption tube is mounted in the flame, and the atoms enter the tube through a hole half-way along its length. Light passes through the tube improving reproducibility and defining the residence of the atoms in the flame. Silica absorption tubes have been used with a considerable increase in lifetime. Figure 9 shows the Delves microsampling cup. This system has been used principally for lead determination in blood and urine samples [36-38]. Accurate results can be obtained from 10 μL of blood, with a sample pretreatment consisting of partial oxidation with hydrogen peroxide. The precision obtained is good and it is possible to analyze about 30 samples per hour because the sample preparation is very short. This system has been used for lead determination in blood samples for some time.

A second limitation of the flame atomizer is due to the nature of the flame.

1) Flames present a background radiation and absorption which consist of bands and continuous spectra. The banded spectra arise from the excited molecules and radicals in the flame gases, and the continuous spectra from the dissociation, ionization and recombination of these species. The analytical line of interest must not be present in a region of high flame background.

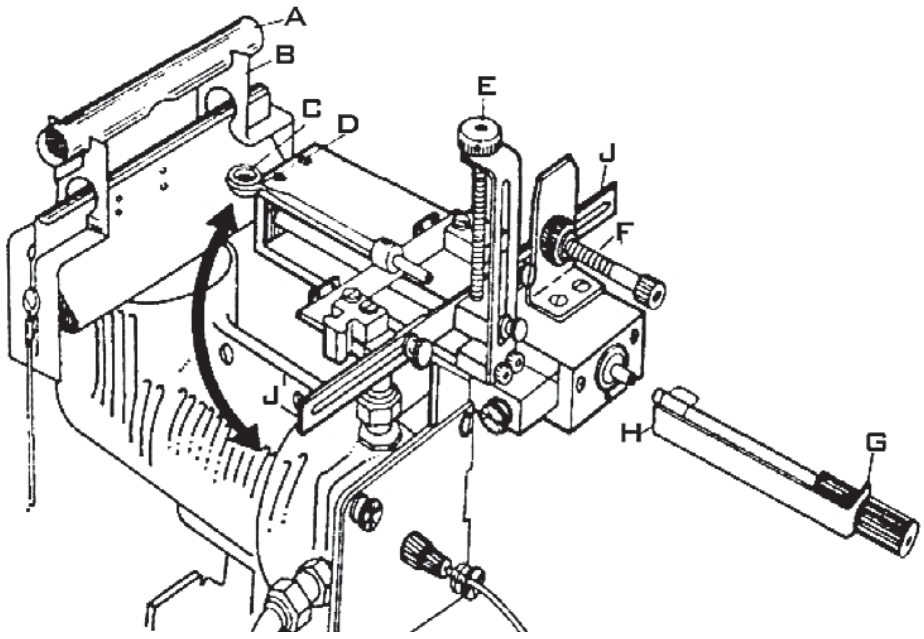


Figure 9. Delves microsampling cup, a) nickel absorption tube, b) nickel support for a), c) nickel crucible, d) platinum-wire holder, e) vertical adjustment screw, f) horizontal angular adjustment, g) adjustment screw for slide stop (h), j and j' rotating limb.

2) Particulate matter in the light path may produce scattering of the radiation.
3) The flame or the high-pressure cylinders used may present a hazard.
4) The flame gas can be expensive and an extraction system is necessary.
5) Flame can be dangerous, explosion hazards are always present with flames of high burning velocity and flame products may be toxic.
6) There is no precise control over species in the flame.

3.2.3. Interferences in FAAS

The interferences in FAAS can be classified as spectral, ionization and chemical interferences.

Spectral interferences caused by direct overlapping of the analytical line emitted by the radiation source and the absorption line of a concomitant element are limited to a few individual cases, for example the interferences of Mn 403.076 nm emission line with Ga 403.298 nm line or the Mg 285.21 nm line with Na 285.28 nm line. But, in general, no real interferences are known on the

main resonance lines, so care is only required with the occasional use of less sensitive alternate lines.

More important are the spectral interferences produced by the overlap of molecular bands and lines, for example the interference of the calcium hydroxide absorption band on the barium line at 553.55 nm, or the interference of alkylhalides. In this case, a matrix such as seawater or urine can produce significant background signals. These interferences caused by the absorption of molecules or radicals can be eliminated by using a background correction system such as the deuterium background correction system.

The ionization interferences of atoms and molecules can occur at high flame temperatures, principally when the oxygen of nitrous oxide was used as the oxidant. In these conditions equilibrium occurs as follows:

$$M \leftrightarrow M^{+} + e^{-}$$

In which M represents a neutral atom and M^{+} its ion. This process is produced principally for elements with low ionization potential such as alkali and alkali earth elements. The presence of the ions instead of neutral atoms produces interference, normally a decrease in the signal, because the atomic spectra are different for the ion than that for the neutral atom. This interference can be avoided if the medium contains not only the species M, but species B as well, and if B ionizes according to the equation

$$B \leftrightarrow B^{+} + e^{-}$$

then the degree of ionization of M will be decreased by the mass-action effect of the electrons formed from B. This element B must have an ionization potential lower than the ionization potential of M, and it is called an ionization suppressor. For example K, with an IP of 4.339 eV can be used as an ionization suppressor in the strontium determination, where IP is 5.692 eV.

In general, in FAAS the chemical interferences are more common than the spectral. The more common type of this interference is due to the presence of anions in the sample, which can form compounds of low volatility with the analyte. The most famous is that of sulphate or phosphate on the calcium signal. These anions produce a decrease in the calcium signal because they form compounds of low volatility. To avoid this interference it is possible to use higher temperatures, but when it is not possible to eliminate the interfering effect, it is possible to use releasing agents. These agents are cations that react preferentially with the interfering anion, avoiding its interaction with the analyte. For example, strontium or lanthanum can be used as releasing agents in the calcium determination. Also, to avoid the formation of compounds of low volatility it is possible to use protective agents, which form stable but volatile species with the analyte. The most common protective agents are

ethylenediaminetetraacetric acid (EDTA), ammonium salt of 1-pyrrolidinecarbodithioic acid (APDC), 8-hydroxyquinoline (oxine).

Association–dissociation equilibria can also be important interferences. In a flame various reversible reactions of dissociation or association can occur. Reactions such as:

$$MO \leftrightarrow M + O$$
$$M(OH) \leftrightarrow M + OH$$

are possible. These reactions may play an important part in the nature of emission or absorption spectra for an element. These oxides or hydroxides in the flame can produce molecular bands that can be more intense than the lines for the atoms. Moreover, the oxygen anions effect of other anions can also produce this type of interference. Thus, the intensity of the Na line decreases in the presence of HCl. This can be explained by the reaction: $NaCl \leftrightarrow Na + Cl$ where the formation of NaCl decreases the Na signals. Another example is the increase of the vanadium signal in the presence of aluminum. This can be explained by assuming that both metals interact with the oxygen:

$$VOx \leftrightarrow V + Ox$$
$$AlOx \leftrightarrow Al + Ox$$

When the aluminum is present in the flame the oxygen concentration is small, thus the presence of aluminum causes the first equilibrium to shift to the right increasing the vanadium concentration and the absorbance signals.

3.2.4. Lead determination by FAAS

Lead is usually determined by FAAS using an air–acetylene flame. The principal resonances lines are show in Table 2.

Although the most sensitive line is 217.0 nm, the line most commonly used is 283.3 nm. This is due to the poor signal-to-noise ratio, and the greater background attenuation effects when using the 217.0 nm line.

Table 2
Principle resonance lines for lead

Line (nm)	Characteristic concentration (mg/L)
217.0	0.08
283.3	0.2
261.4	5
368.4	17
364.0	40

Aluminum, beryllium, zirconium, phosphate and sulphate can be interferences in the lead determination, but they can be controlled by adding EDTA as a protective agent [39]. Nevertheless, large amounts of other ions such as iron can produce interferences. Thus 10.000 μg/mL of iron can increase the response of 5 ppm of lead by 35% in an air-acetylene flame [40].

The detection limits obtained in the lead determination by flame are in the order of 0.01 mg/L. These limits can be improved using the discrete sample nebulization technique [27], the sampling boat [41] or the Delves sampling cup [35].

3.2.5. Electrothermal atomization (ETAAS)

To avoid the most important limitations of the flame atomization such as the low efficiency of the nebulization process and the short residence time of the atoms in the flame, the electrothermal atomizer (ETA) has been developed. This atomizer uses an electrically heated refractory material on which the sample is deposited obtaining a transient formation of free atoms. Although there are different forms of these atomizers, the most commonly used is a cylindrical tube 25-50 mm long and 5-10 mm in diameter. The tube, usually named furnace, is heated by low voltage (10 V) and high current (up to 500 A) from a well stabilized step-down transformer. The material of the furnace must have a high electrical conductivity and must exhibit high thermal resistance and a high durability. Although materials such as W or Ta have been used in electrothermal atomization because their melting temperature is higher than 2600°C, these materials present some problems such as their extreme brightness at high temperatures. The most common material used today is graphite. The two major disadvantages of graphite are its porosity and its tendency to form carbide, but these problems can be partially overcome by coating the tube with pyrolytic graphite which is far less porous. The tubes coated with pyrolytic graphite minimize losses by diffusion of the atoms into the porous substrate, which improves the atomization process of many elements. A scheme of an electrothermal atomizer is shown in Figure 10.

The graphite tube is open at both ends and has a central hole for sample introduction with an autosampler. The tube is in contact with a pair of cylindrical graphite electrical contacts located at both ends. These contacts are refrigerated with water. Two inert gas streams, usually argon gas, are provided. An external gas stream prevents the entrance of outside air and the possible tube destruction and an internal stream flows into both ends of the tube and out of the central sample port. This gas transports vapors generated during the mineralization of the sample. A typical electrothermal program has three steps: drying, ashing and atomization. The first step, drying, is to evaporate the solvent of the sample. For aqueous solutions, a temperature slightly above 100°C is used with a drying time that depends on the sample amount. The second step is

ashing (pyrolysis or charring) of the solid residue remaining after the drying step; at the end of this step all the organic or inorganic matrix must be destroyed.

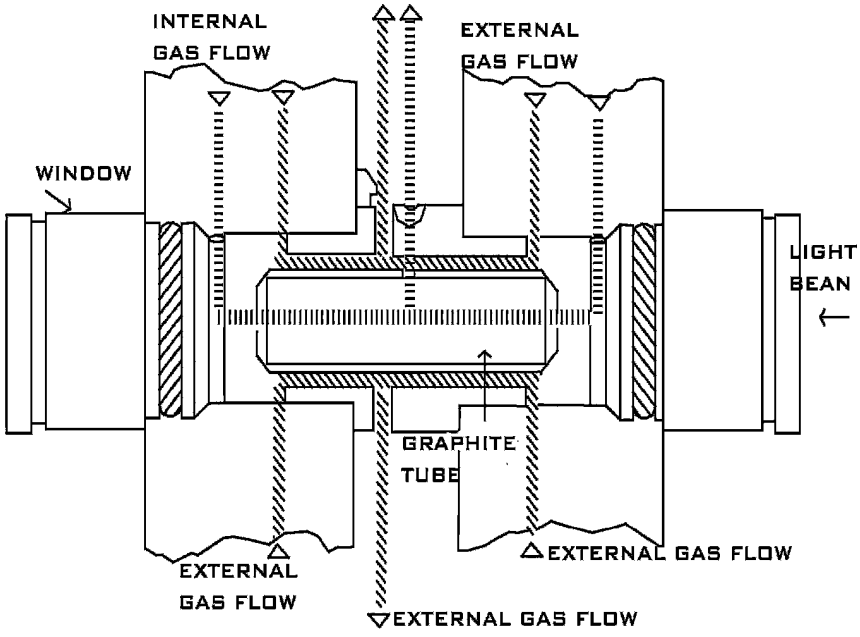


Figure 10. Scheme of a graphite furnace.

The temperatures used in this step are between 300-1500°C depending on the analyte and the sample matrix. During this step, an alternative gas, oxygen or air, can be introduced into the tube to achieve a better decomposition of the matrix. When this step is finished the residue should consist only of the analyte in a molecular form and the minimum amount of the inorganic matrix that was not destroyed at the temperatures used in this step. To avoid losses of sample between the drying and the ashing step, a temperature ramp of some 20-30 s (depending of the sample matrix) is introduced before the ashing step. The third step is atomization in which the dissociation of the analyte molecular species occurs at a higher temperature (1200-2900°C) and a transient formation of free atoms is then obtained. In order to avoid losses of the free atoms of the analyte before atomization, a rapid increase in temperature must be used (200°C/s). During this step, the flow of the internal gas is stopped in order to extend the residence time of the atoms and consequently to increase sensitivity. Table 3 show a typical program of an electrothermal atomizer performing lead determination in a water sample. The temperatures and the duration of each step can be optimized for a given element and matrix.

Table 3
Program for lead determination in water, with Pd-$Mg(NO_3)_2$ matrix modifier

Step	Temperature °C	Ramp time S	Hold time s	Ar flow mL/min
Dry	110	20	10	300
Pyrolysis	900	20	10	300
Atomization	1800	0	3	0
Cleaning	2000	1	3	300

A modification of the graphite tubes has been the introduction of the L'Vov platform [42,43]. This is a small graphite platform located beneath the sample entrance port. The sample is evaporated and ashed on this platform in the usual way. The platform is heated by the radiation from the wall of the tube so that the increase in the sample temperature is delayed with that of the wall and the gaseous phase. The sample atomization occurs when the gaseous phase has reached a temperature plateau. For volatile elements an enhancement of sample dissociation is obtained, the interference effects are minimized and more reproducible peaks are obtained.

There are two modes of furnace heating: the longitudinal heated mode and the transverse heated mode [44]. The transverse heated mode has been developed to reduce the temperature gradient between the tube ends and the tube center observed with the longitudinal heated mode. This temperature gradient produces undesirable effects during atomization, e.g., recondensation and memory effects principally for refractory elements. The transverse heated tubes are shorter that the longitudinal heated tubes, then a decrease in the sensitivity is observed due to more loss of atomic vapor. In order to increase the sensitivity end caps can then be used.

The most important advantages of the electrothermal atomizer over the flame atomizer are the increase in the residence time of the free atoms compared with the flame, the high efficiency of the atomization process, 100% (all sample is atomized), the use of an inner atmosphere in the system which avoids interferences, and the possibility to control the different steps before sample atomization.

3.2.6. Interferences in ETAAS

The interferences in electrothermal atomization atomic absorption spectrometry are classified in two groups: spectral interferences and non-spectral interferences.

3.2.6.1. Spectral interferences

These interferences, due to the incomplete isolation of the absorber radiation, are as follows:

The overlapping of the atomic lines: this interference is not very important because the number of absorption lines is not very high, and because, in general, the absorption lines are narrow. The possible effect of these interferences depends on the overlapping of the lines and of the concentration on the interferent element. To control this interference it is possible to use an alternative line or to perform the exact measurement of the blank and to subtract that from the analytical measurement.

The scattering of the radiation by particles: this interference depends on the number of particles by volume, the wavelength and the particle size. To reduce the effect of this interference the sample amount can be decreased or the optimum position of the atomizer looked for, thus avoiding the scattered radiation following the detector.

The absorption of foreign radiation if the source emits other lines: this interference can be avoided using an adequate monochromator.

The background absorption, this is the most important type of interference in ETAAS: the background absorption can be produced by absorption of the present molecules, the smoke and emission of light by the atomizer. To control this interference it is necessary to use a background correction system such as the continuous background correction system. In this system a deuterium lamp for the UV zone and tungsten lamp for the visible zone are introduced in the spectrometer. The deuterium lamp provides a continuous radiation throughout the UV region and the W lamp provides a continuous radiation for the VIS region. Figure 11 shows a scheme of a continuous-source background correction system.

The radiation of the hollow cathode lamp and the radiation of the deuterium or W lamp are passed alternately through the graphite atomizer using a rotating chopper. When the radiation of the hollow cathode lamp (HCl) or the electrodeless lamp (EDL) passes through the sample, the detector measures the atomic absorption signal and the background absorption signal, but when the radiation of the deuterium or W lamps passes, only the background absorption signal is measured because the slit width is kept sufficiently wide so that the fractions of the continuous source that are absorbed by the atoms of the sample is negligible. The absorbance of both lamps is then subtracted to obtain only the atomic absorption signal. This correction system presents some disadvantages leading to over-corrections in some systems and under-corrections in others. Perhaps the most important limitations are the degradation of the signal-to-noise ratio due to the presence of a new lamp and the chopper.

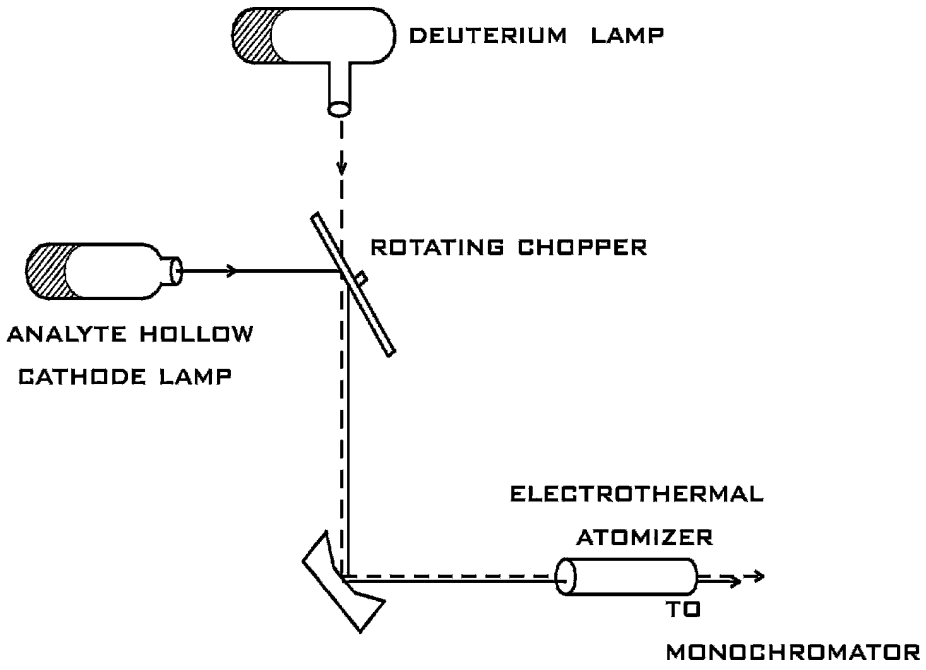


Figure 11. Scheme of a continuous-source background correction system.

Another procedure for correction of the background absorption is the use of the Zeeman background correction system. This system is based on the Zeeman effect on atomic energy levels. When an atomic vapor is exposed to a magnetic field, splitting of energy levels of the atoms is produced, leading to the formation of several absorption lines for each electronic transition (Figure 12).

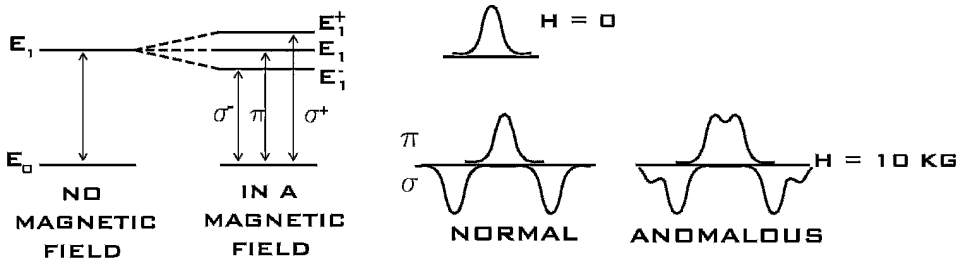


Figure 12. Principle of the Zeeman effect.

The simplest splitting pattern (normal Zeeman) leads to a central or π line, which corresponds with the wavelength of the line without magnetic field, and two equally spaced, σ lines, which present a wavelength with little difference. The π line has an absorbance that is twice that of each σ line. The sum of the absorbance for the lines is equal to that of the original line. Depending on the atom, other splitting patterns, known as anomalous Zeeman, are possible. The π component is absorbed by both the analyte and the background absorbing species, while the σ components are only absorbed by the background absorbing species. Through the use of polarizers and by modulating the magnetic field, it is possible to obtain the background subtraction. The magnetic field can be applied to the radiation source (direct Zeeman effect) or to the atomizer (inverse Zeeman effect). The magnetic field can be characterized by its mode, transverse or longitudinal, or by its frequency, dc (permanent magnet) or ac (up to 12 Hz). In the transverse mode the magnetic field is applied perpendicularly to the beam whereas in the longitudinal mode it is applied parallel to the beam, then the π component is not observed and the polarizers are not necessary.

The most important advantage of this correction system is that it corrects the structural background absorption because the background measurement is performed exactly at the analytical wavelength; moreover it is effective for any wavelength. However, it presents some limitations, principally a slight decrease in the sensitivity and a possible rollover of the calibration curves.

3.2.6.2. Non spectral interferences

The main non-spectral interferences are:

Physical interferences, which depend on the alterations in the physical properties of the sample that can produce difficulties in sample introduction into the graphite tube. To avoid these interferences the use of surfactant agents such as Triton X-100 is carried out. Also, the integration of the signal in the mode peak area and the use of heating ramps are also recommended.

Memory effects, which are due to an incomplete atomization of the analyte and are important for refractory elements such as V or Mo. To avoid this problem the introduction of a cleaning step at the end of the heating programme is recommended.

Chemical interferences, which can arise from the losses of the analyte as a volatile salt. This interference is important when halides are present in the sample, at the ashing or atomization stage at temperatures too low to reach atomization, and can lead to losses, for example of $CaCl_2$ or $PbCl_2$. To avoid this interference the use of the hydrochloric acid for sample dissolution should be avoided. When this is not possible, the use of **chemical modifiers** is recommended. The objective when using these chemical modifiers is to increase the difference in volatilization between the analyte and the matrix to obtain, for instance, a less volatile analyte (analyte modifier) or a more volatile matrix

(matrix modifier). Modifiers are usually in the form of liquid inorganic salts such as $Pd(NO_3)_2$, $Ni(NO_3)_2$, $Mg(NO_3)_2$, NH_4NO_3, $NH_4H_2PO_4$ etc. Usually, it is necessary to look for a chemical modifier which is adequate for each analyte and for each matrix. In order to simplify this, the use of an universal matrix modifier has been proposed. The mixture palladium-magnesium nitrate, is adequate for most volatile and medium volatile elements that can be determined with graphite furnace [45,46].

The mechanism of stabilization of this modifier is not exactly known but is probably due to the formation of intermetallic species between palladium, magnesium and the analyte, as these species are stable at higher temperatures. In other situations it can be necessary to change the chemical composition of the sample matrix, principally if the matrix contains a chloride ion, because this ion forms volatile species that are lost before atomisation. This is very important in matrices such as seawater or saline mediums. In these cases, the use of ammonium nitrate is proposed as matrix modifier. With this modifier the reaction is:

$$NaCl + NH_4NO_3 \Rightarrow NH_4Cl + NaNO_3$$

which transforms NaCl (boiling point 1413°C) into sodium nitrate (decomposes at 380°C) and ammonium chloride (sublimes at 335°C). Another possible problem is the reaction of the sample with the surface of the graphite tube. To avoid this we can use pyrolytic graphite tubes, but in some cases this is not sufficient. Then, it is possible to coat the surface of the tube with chemical modifiers. To this end, noble metals such as Pt, Ir, Zr, W, etc., have been proposed as permanent chemical modifiers to avoid contact between the sample and the graphite tube [47].

Chemical interferences can be also be produced by anions and cations. To control these interferences it is necessary to use the standard addition method and to compare this with aqueous standard calibration. When both graphs are not parallel an interference is present, therefore the standard addition method must be used for calibration. Finally, some elements can react with the surface of the graphite tube and form carbides that are difficult to atomize. Rapid heating and a reproducible surface help to reduce the problem, as well as the coating of the tube, for example with La, W, Zr, etc.

3.2.7. The stabilized temperature platform furnace (STPF)

The concept of STPF was introduced by W. Slavin [48] in 1981. This concept consists of a series of conditions that must be used to obtain an isothermal furnace. These conditions include:

- the use of the L'Vov platform in each graphite tube (retardation of sample atomization)
- a rapid heating rate for graphite tube (1500-2000°C/s), because by increasing the heating rate, the platform effect is enhanced
- shutdown the forced gas stream through the graphite tube during atomization (stopped flow)
- fast-reacting electronics to register the fast signals without distortion
- integration of the peak area (integrated absorbance)
- use of graphite tubes with a good pyrolytic graphite coating
- use of a chemical modifier
- use of a powerful background corrector (Zeeman effect)

The introduction of the STPF concept made ETAAS a highly sensitive, reliable and versatile technique for trace and ultratrace analysis in a wide range of matrices. With the use of the STFP concept, it is possible to achieve three aims of interference-free determination in three steps:

1. To separate concomitants as far as possible prior to atomization
2. To control the reactions in the condensed phase to prevent analyte losses and to better separate concomitants
3. To minimize the influence of non-separated interferents on the analyte in the gas phase by as complete atomization as possible.

The use of the STPF conditions is always recommended in order to obtain interference-free methods

3.2.8. Lead determination by ETAAS

In the graphite furnace, the second resonance line for Pb at 283.3 nm is normally used. The line 217.0 nm has about twice the sensitivity but the energy of the line source is difficult to balance against the D_2 source at 217.0 nm. When Zeeman background corrector is used, the 217.0 nm line gives better detection limits. The characteristic mass at the 283.3 nm line on the platform was about 5 pg /0.0044 A.S.

Three different atomization mechanisms have been published for lead atomization in a graphite furnace:

1. the dissociation of the condensed phase of PbO(s)
2. the dissociation of the gaseous phase of PbO(g) and
3. the direct vaporization of the reduced metal

The first mechanism [49] proposes that the $Pb_{(g)}$ atoms can be the result of the thermic dissociation of the $PbO_{(s)}$. The second mechanism proposes the dissociation of $PbO_{(g)}$ to give $Pb_{(g)}$ and oxygen, this mechanism has been proposed after a study carried out using mass spectrometry detecting $PbO_{(g)}$ and $Pb_{(g)}$ [50]. In the third mechanism the reaction between the PbO and the surface of the graphite tube obtaining $CO_{(g)}$ and $Pb_{(g)}$ is possible.

Matrix modifiers have been shown to be important for the successful determination of lead in many matrices. Most workers use $NH_4H_2PO_4$ or

$(NH_4)_2HPO_4$ [51-55], there being no difference in the effectiveness of these compounds. Some authors [56] investigated the stabilization of lead by phosphate. In this case they found no signal for $PbO_{(g)}$, and free $Pb_{(g)}$ appeared at 1150K together with signals for PbO and PbO_2. The authors concluded from this that a thermal decomposition of a lead phosphate took place in the condensed phase. In many cases it is preferable to use a mixture of $Mg(NO_3)_2$ and the phosphate [53]; this modifier has been used for different matrices such as water, food, blood, etc.

Other authors used La as La_2O_3 [56] or as the chloride [57] as a matrix modifier for Pb, but La has also been used to coat the graphite tube surface [58], thus a reduction in interferences has been observed by treating the graphite tube with La. To coat the tubes, solutions of lanthanum nitrate hexahydrate were first heated to high temperature in the tube, forming a layer of lanthanum carbide on the tube surface.

NH_4NO_3 has been proposed for lead determination in blood, urine and seawater, thus avoiding problems due to high saline concentrations [59,60]. Oxalic acid has been proposed as a matrix modifier because the oxalic acid decomposed completely to CO_2 below 300°C and when oxalic acid and PbO were heated together in an inert atmosphere at 300°C, free Pb was produced; this acid can remove the characteristic interference of 0.1% $MgCl_2$ on the Pb absorption signal.

Tube treatment with a carbide-forming metal has often been shown to be helpful for the determination of lead. Moreover, the lanthanum cited before, the Zr-treated tubes [62] or the Mo treated tubes [63] have also been proposed. On the other hand, the universal chemical modifier, the mixture Pd-$Mg(NO_3)_2$ with the use of the conditions of STPF has been used as a chemical modifier for lead determination in different matrices obtaining good results.

3.3. Atomic emission spectrometry (AES)

Atomic emission spectrometry is based on the measurement of the energy emitted during a deexcitation process of electrons produced by a transition between excited levels and lower or ground levels. This energy is specific for each element and the use of an adequate wavelength selection system allows the verification of the presence of this element and the determination of its concentration. An atomic emission spectrometer consists of an atomization system, a sample introduction module, a dispersion system, a detector and a system for data acquisition and processing.

Among the different atomic emission techniques (flame, arc, spark, or plasma), the most used in routine analysis today is atomic emission based on plasma sources.

A plasma is an ionized gas, a gaseous mixture containing a significant concentration of cations and electrons (with a net charge close to zero). The

more common plasma used in atomic emission is argon plasma. This plasma contain Ar ions and electrons as the conducting species. The argon ions can absorb power from an external source to obtain high temperatures. At these temperatures (about 1000K) further ionization processes sustain the plasma, which, in turn, will transmit part of this energy to the sample to atomize and excite it.

According to the electric field used to create and sustain the plasma, plasmas can be classified as: *Direct current plasma, DCP* (a direct field is established across electrodes), *Inductively coupled plasma, ICP* (a high-radio frequency field is applied through a coil) and *Microwave induced plasma, MIP* (a microwave field is applied). The inductively coupled plasma (ICP) source offer the best advantages, principally the best sensitivity and freedom from interferences [64,65].

Figure 13 shows the scheme of a typical instrument of ICP-AES. The sample, usually a liquid sample, is converted in an aerosol which is transported to the plasma where it is desolvated, vaporized, atomized and excited by the plasma. The excited atoms or ions emit their characteristic radiation which is introduced into a spectrometer where the wavelength for measurement is selected, detected and transformed into electronic signals which are converted into concentration information.

3.3.1. ICP

The inductively coupled plasma source is called the torch. This torch contains three concentric quarzt tubes through which argon flow. The external tube has a diameter of about 2.5 cm and surrounding the top of this tube is the induction coil to which the power of the radio-frequency generator is applied. The ionization of the argon is initiated by a spark from the tesla coil. The ions and electrons formed interact with the magnetic field and, as a consequence, they flow in a close annular path, then an ohmic heating is the consequence of their resistance to this movement. Due to the high temperatures obtained in the plasma, it is necessary to use a refrigeration system to avoid damage to the torch. For this, a tangential argon flow is introduced for the medium tube. This flow cools the inside walls of the central tube and the center of the plasma radially. The central tube of the torch is used to introduce the aerosol of the sample with argon gas.

3.3.2. Sample introduction

The sample is introduced with a nebulizer. Nebulizers are devices that convert the liquid sample into an aerosol that can be transported to the plasma. The nebulization process is one of the critical steps in ICP-AES, therefore different types of nebulizers have been developed. The nebulizers used in ICP are based on pneumatic forces and ultrasonic mechanical forces.

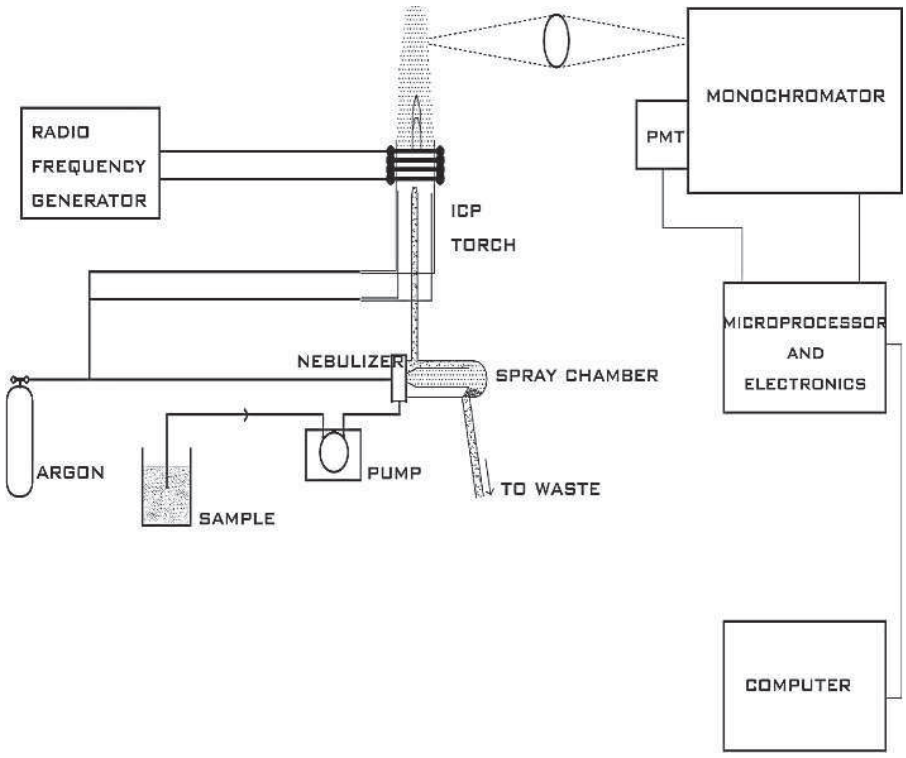


Figure 13. Major components of a typical ICP-AES instrument.

Three different types of pneumatic nebulizer can be used. The first is the concentric nebulizer, used also in FAAS. In this nebulizer, the solution is introduced through a capillary tube to a low-pressure region created by a gas flowing rapidly past the end of the capillary. The low pressure and high speed gas combine to break up the solution into an aerosol. The most important problem with this nebulizer is that of clogging, but advances in its design have improved their tolerance to dissolved solids up to solid concentrations of 20% NaCl. The second type of pneumatic nebulizer is the cross-flow nebulizer, a high speed stream of argon gas directed perperdicular to the tip of a capillary tube containing the sample. This nebulizer is less efficient than the concentric nebulizer for creating the small droplets needed for ICP, but its design minimizes the clogging problems. The third type of pneumatic nebulizer is the Babington nebulizer which was developed to nebulize fuel oil for industrial burners. In this nebulizer the liquid flows over a smooth surface with a small

hole in it. High speed argon gas emanating from the hole shears the sheet of liquid into small drops. This nebulizer is less sensitive to clogging and can nebulize very viscous liquids.

In the ultrasonic nebulizer the liquid sample is pumped into an oscillating piezoelectric transducer; the oscillations break the sample into a fine aerosol; the formation of the aerosol is independent of gas flow. The efficiency of this nebulizer is at least 10-fold greater than typical pneumatic nebulizers in obtaining better detection limits. The use of this nebulizer increases the water load to the ICP; it is then necessary to introduce a desolvation unit.

After the aerosol is created by the nebulizer, it must be transported to the torch and injected into the plasma, but only the very small droplets of the aerosol are adequate for injection into the plasma. To separate the droplets according to their size, a spray chamber (similar to that used in FAAS) is placed between the nebulizer and the torch. The primary function of this spray chamber is to remove large droplets from the aerosol; a second function is to smooth out pulses that can occur during the nebulization process due to the pumping of the solution. In general, droplets with diameters of about 10 μm or smaller pass into the plasma (normally this represents between 1 and 5% of the sample) and the rest of the sample (95-99%) is drained into a waste container.

The introduction of the sample can be carried out using alternative sample introduction techniques. The most widely used alternative technique is hydride generation. In this technique, the sample in a diluted acid media is mixed with a reducing agent, such as sodium borohydride in dilute sodium hydroxide. This reaction produces atomic hydrogen that reacts with some elements such as Hg, Sb, As, Bi, Ge, Pb, Se, Te and Sn in the solution to form volatile hydrides of these elements. These hydrides are separated in a gas-liquid separator and transported to the plasma. Using this system of sample introduction an improvement in the detection limits by a factor up to 1000 can be obtained. This is due to two reasons: 1) the sample introduction rate for the hydride if often as much as ten times the rate of a pneumatic nebulizer; and 2) the efficiency of the hydrides delivered to the plasma is near 100%.

Electrothermal atomizers, principally the graphite furnace, have been used to vaporize a small portion of a liquid or solid sample. In these systems the normal sample introduction system of the ICP instrument is replaced by a graphite furnace. The sample vapor from the furnace is introduced into the center of the ICP discharge in a conventional torch. The efficiency of the transport is 100%, thus high sensitivity is obtained. Nevertheless, some aspects, such as the necessity to compromise furnace conditions in line with the ICP, have limited the number of applications. Due to the non-continuous nature of the electrothermal atomization, it is necessary to use an ICP instrument that can record transient signals. In the ICP-AES instrument the introduction of solid samples via arc and spark sources or by laser ablation is possible. In the laser ablation technique a high-power laser is used to vaporize a small portion of the

sample. The difficulty of standardizing with solids and the high cost have limited the use of this technique.

3.3.3. Plasma emission spectrometers

There are two types of plasma emission spectrometer: sequential and simultaneous multichannel.

The sequential instrument is less complex and measures the line intensities on a one-by-one basis. This instrument can usually be used for emission analysis of an ICP source or for atomic absorption with flame or graphite furnace. To change from emission to absorption mode, only the movement of the movable mirrors is necessary. Selection of the wavelength is carried out by a holographic grating driven by a motor, obtaining a change of 0.007 nm. Normally, there are two gratings, one for the UV region and another for the VIS region. The detection system is a photomultiplier tube. With this type of spectrometer it is possible to determine three or four elements per minute.

In the multichannel instrument numerous photomultipliers are located behind fixed slits along the curved focal plane of a concave grating monochromator. In these instruments the entrance slit, the exit slit of the monochromator and the grating surface are located along the circumference of a Rowland circle, the curvature of which corresponds with the focal curve of the concave grating.

In addition to the classical dispersing device, the diffraction grating, there is another optical component, the prism, which disperses polychromatic radiation into its characteristic wavelengths. In recent years, the use of two dispersing systems such as a diffraction grating and a prism or two diffraction gratings has been proposed. The two optical components are positioned perpendicular to each other. The echelle grating separates the polychromatic radiation by wavelength and produces multiple, overlapping spectral orders. The second dispersing device separates the overlapping orders into a two-dimensional pattern. These systems present some advantages over conventional spectrometers. The optics result in a very good efficiency level in each of the spectral orders and the system has excellent resolution.

With reference to the detector, in recent years the advanced solid state detectors such as the photodiode array (PDA), the charge-injection device (CID), and the charge-coupled device (CCD) are the more widely used. The CID and CCD devices are based on the light-sensitive properties of solid-state silicon and belong to the broad class of silicon-based devices called charge transfer devices (CTD).

A CTD detector is based on the use of a block of very high purity of crystalline silicon with an insulating layer of SiO_2. The silicon-silicon bond may be broken by photons and then an electron is released and a hole is formed in the crystalline structure. When a voltage is applied, the free electrons will move in

the opposite direction of the applied electric field or toward the silico-silicon dioxide interface while the holes will move in the other direction or in the same direction as the electric field and leave a region depleted of positive charge. The movement of electrons and holes creates a current proportional to the amount of photons impinging on the structure. The CTD elements, known as pixels, are arranged in a two-dimensional silicon wafer configuration from 512×512 to 4096×4096 pixels. Each pixel is capable of storing photon generated charge. A typical ICP spectrometer with a CID detector used an echelle design and a CID detector with over 250,000 pixels capable of detecting ICP spectral lines across a large wavelength region.

3.3.4. Interferences

The most important problem in the effective use of inductively coupled plasma-atomic emission spectrometry is that of spectral interferences. These interferences are more important than in other emission sources, such as flames, arcs or sparks, because emission lines that might be expected to be weak are quite intense. The origin of the spectral interferences can be due to the inherent argon spectrum (line and continuum) or spectral features (line and continuum) generated from molecules or atomic species introduced into the argon plasma. The severity of the interference depends on the wavelength proximity of the non analyte feature to the analyte line, on the spectral distribution of the feature, and on the intensity and stability of the interfering signal [66].

Spectral interferences are classified in four categories: simple background shift, sloping background shift, direct spectral overlap and complex background shift

3.3.4.1. Simple background shift

This is the most common interference in ICP-AES and the easiest to correct. It is a shift in background intensity that is essentially constant over a given range, or on either side of the analyte line, the shift moves up or down. Figure 14(a) shows the simple background shift caused by 1000 mg/L aluminum on the tungsten 207.911 line. The effect of the aluminum is to increase the background emission level on the tungsten line, thus producing a positive error. But the intensity of the aluminum continuum can be automatically measured and then subtracted from the tungsten intensity. To do this, a background correction point would be selected close to the profile of the tungsten line.

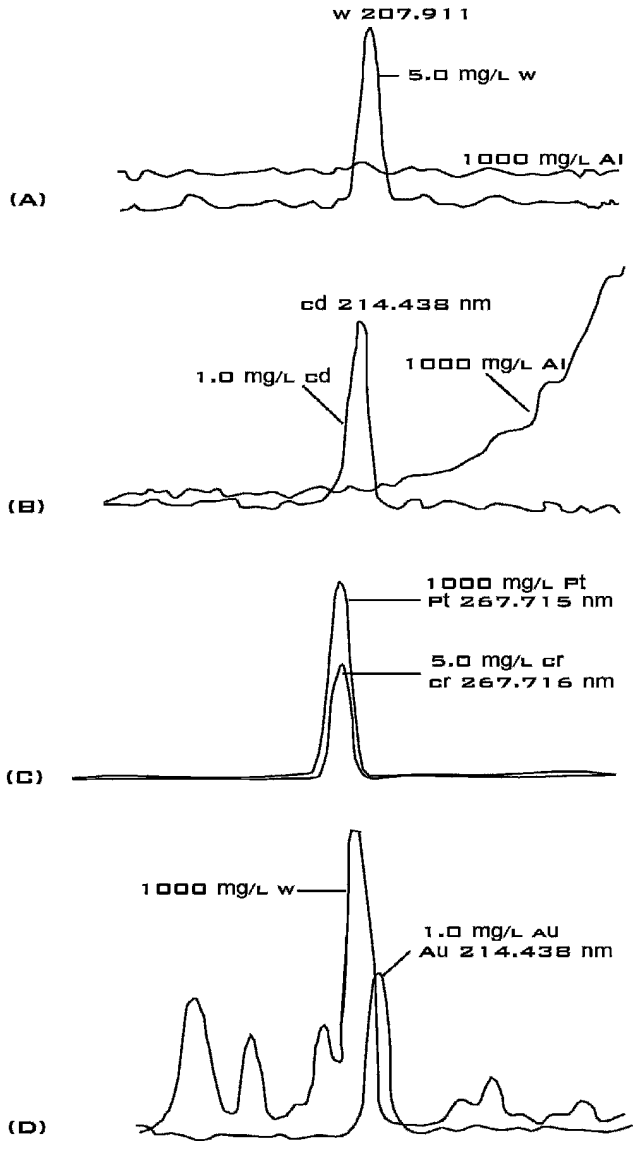


Figure 14. Spectral interferences in ICP-AES. (a) Simple background shift caused by 1000 mg/L Al on the W 207.911 line. (b) Al background spectrum showing a sloping background shift for the Cd line. (c) Direct spectral overlap caused by a Pt matrix for Cr at 267.716 nm. (d) W matrix spectrum causing a complex background shift for Au at 267.595 nm.

3.3.4.2. Sloping background shift

Figure 14(b) shows an example of sloping background shift produced for 1000 mg/L of aluminum on the cadmium line at 214.438 nm. This overlap produces an upward sloping positive background shift at the cadmium wavelength. The cause of this interference is usually the presence of a very intense atomic or ionic emission line that has been broadened by a high concentration of the element in the sample or by the presence of electric fields in the plasma. To correct this interference it is necessary to use two background correction points on either side of the cadmium line.

3.3.4.3. Direct spectral overlap

This interference is produced when two elements have exactly the same wavelength. Figure 14(c) shows the direct spectral overlap caused by a platinum matrix on the chromium line. The use of the high resolution optical system limits the effect of spectral overlap, however it is not always possible to avoid this effect. To avoid this interference, it is best to use an alternate line for the analysis, but if this is not possible, a technique called interelement correction (IEC) can be used. In this technique, the contribution of the interfering element is corrected by measuring the emission intensity of the interfering element at another wavelength and applying a correction factor to the results.

3.3.4.4. Complex background shift

In this interference a shift in background intensity varies on either side of the analyte line. In Figure 14(d) we can see the interference of 1000 mg/L of W on the gold line. This interference is produced by the occurrence of a number of intense emission lines, and perhaps directly overlapping the analyte wavelength. Although it is possible to use the automatic background correction systems available in the spectrometer, it is recommended that an alternate wavelength for the element be used to correct this interference.

3.3.4.5. The absorption interference

This interference is produced when part of the emission from an analyte is absorbed before it reaches the detector. This is due to two causes. The first is the absorption of emission below 190 nm by the oxygen in a non-purged spectrometer. The second cause is when the concentration of a strongly emitting element is so high that the atoms or ions of that element that are in the lower energy state absorb significant amounts of the radiation emitted by the excited atoms or ions. This absorption, called self-absorption, usually determines the upper end of the linear working range for a given emission line.

3.3.5. Lead determination by ICP-AES

Lead determination by ICP-AES can be carried out at the wavelength indicated in Table 4 where we can see the detection limits obtained for each wavelength as well as the BEC (background equivalent concentration). The BEC is defined as the concentration of a solution that results in an analyte emission signal equivalent in intensity to that of the background emission signal at the measurement wavelength.

Table 4
Lines, detection limits and BECs for lead determination by ICP-AES

Wavelength	Detection limit (mg/L)	BEC (mg/L)
220.353	0.0420	1.43
217.000	0.0900	3.03
261.418	0.1300	4.35
283.306	0.1420	4.76
224.688	0.3330	11.11
405.781	0.2720	9.09

With reference to possible interferences, the lines 220.353, 261.418 and 405.781 are interference free lines for lead determination. On line 217.000 nm there is an interference of the 217.582 nm antimony line. For line 283.306 nm there are three important interferences: tin interference at 283.998 nm (intensity of 90), copper at 283.563 (this is the most important interference with an intensity about 3700), and thorium at 283.503 nm (with an intensity of 720). For line 224.688 there is an interference of copper with a line at 224.700 nm.

3.4. Inductively coupled atomic mass spectrometry (ICP-MS)

3.4.1. General aspects

Inductively coupled atomic mass spectrometry has some advantages over atomic optical spectrometric methods, such as:

a) detection limits two or three orders of magnitude better than those obtained with optical methods,
b) the mass spectra are simple and easily interpretable, and
c) the possibility to measure atomic isotopic ratios.

The analysis is carried out in four steps: 1) atomization, 2) conversion of atoms into a stream of ions, 3) the separation of ions on the basis of their mass/charge ratio and 4) counting of the number of ions. The majority of the ions formed are singly charged; then m/z is usually the simple mass of the ion. The two first steps, atomization and conversion of ions, are similar to these steps used in atomic optical spectrometry.

The components of a Mass Spectrometer are an inlet system to introduce the sample, an ion source, the mass analyzer, the detector of ions and the signal processor. The ion source, the mass analyzer and the ions transducer are in a high vacuum zone (10^{-5} to 10^{-8} torr). The function of the mass analyzer is equivalent to the monochromator in an optical spectrometer, but the dispersion is based upon the mass-to-charge ratio of analyte ions.

According to the system for sample introduction and the ion sources there are different types of atomic mass spectrometers. Table 5 shows the most common atomic mass spectrometers [67].

Of these different techniques, we will study inductively coupled plasma-mass spectrometry, ICP-MS. Figure 15 shows a diagram of a typical ICP-MS instrument. As we commented before, the system of sample introduction is similar to the use in ICP-OES. The sample introduction is carried out using a nebulization system for liquid samples or alternative systems for gaseous samples such as the hydride generation system or the electrothermal vaporization for solid samples.

Table 5
Types of Atomic Mass Spectrometry

Name	Atomic ion source
Inductively Coupled Plasma, ICP-MS	High temperature argon plasma
Direct Current Plasma, DCPMS	High temperature argon plasma
Microwave Induced Plasma, MIPMS	High temperature argon plasma
Spark Source, SSMS	Radio frequency electric spark
Thermal Ionization, TIMS	Electrically heated plasma
Glow Discharge, GDMS	Glow-Discharge plasma
Laser Microprobe, LMMS	Focused laser beam
Secondary Ions, SIMS	Accelerated ion bombardment

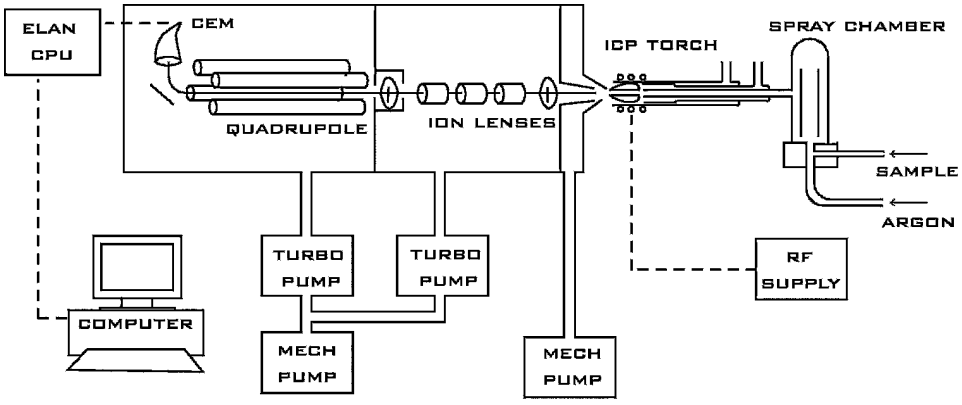


Figure 15. Diagram of an ICP-MS.

To produce the ions, a quartz torch similar to the torch of the conventional ICP-OES is used.

An important part of the instrument is the interface between the ICP torch, which operates at atmospheric pressure, and the mass spectrometer, which operates at a pressure less than 10^{-4} torr. The interface consists of two cones: the first cone, which is called the sampling cone, and a water-cooled nickel cone with a small orifice < 1.0 mm in its center. The plasma passes through this cone and then to a region that is at a pressure of about 1 torr with a mechanical pump; a rapid expansion of the gas is then produced and the plasma is cooled. After the plasma passes through a second cone, called the skimmer, it is introduced into a chamber that is at the pressure of the mass spectrometer, 10^{-4} torr. Applying a negative potential, the separation of positive ions from electrons and molecular species is produced, after that the ions are accelerated and focused by a magnetic ion lens onto the entrance orifice of a mass analyzer. The principal types of mass analyzer are the quadrupole mass analyzer, the time-of-flight mass analyzer and the double focusing analyzer, but the most common type used is the quadrupole mass analyzer.

A quadrupole mass analyzer consists of four parallel cylindrical rods which serve as electrodes. These rods are connected electrically in pairs, one pair to the positive side of a variable dc source and the other pair to the negative terminal. Variable ac potentials, 180 degrees out of phase are applied to each pair of rods. To obtain mass spectra, ions are accelerated into the space between the rods with a potential of 5-10 v. The ac and dc voltages are increased but maintaining their constant ratio. The majority of the ions, except those having a certain m/z value, strike the rods and are converted into neutral molecules; only ions with a range of m/z values can cross the rods and reach the detector. The separation of the ions is obtained in function of their m/z ratio. Normally, the resolution capacity of a quadrupole is about one unit of mass.

The most common detector used with the mass spectrometer is the electron multiplier. These systems are very like the photomultiplier tube used for UV-VIS radiation; they consist of different dynodes at a successively higher voltage to accelerate the electrons. Systems with 20 dynodes produce a current gain of 10^7. A different system is a continuous-dynode electron multiplier, which is a trumpet-shaped device made of glass that is doped with lead and a potential is impressed across the length of the detector. When ions enter into the detector and strike the surface they eject electrons than then skip along the surface ejecting more electrons with each impact. A current gain of 10^5-10^8 can be achieved.

3.4.2. Interferences in ICP-MS

An advantage of the mass spectra obtained with an ICP- MS compared with spectra obtained with ICP-OES is that the mass spectra are usually much

simpler and easier to interpret that the corresponding optical spectra. This is particularly true in the case of rare earths that have thousands of emission lines. But the simplicity of the mass spectra does not mean that the ICP-MS will be an interference-free method. In ICP-MS there are spectroscopic interferences and matrix effects [68]. The spectroscopic interferences are: isobaric interferences, polyatomic ion interferences, and oxide and hydroxide species interferences.

An isobaric interference is produced when two elements have isotopes with the same mass. However, most elements have one, two or three different isotopes, and it is therefore possible to select one isotope without isobaric interference, although this isotope must not be the most abundant. These isobaric interferences are exactly predictable from abundance tables and it is possible to perform corrections using the appropriate software. For example, in lead measurement, ^{204}Hg can interfere with ^{204}Pb.

The polyatomic species formed from interaction between species in the plasma and species in the matrix or the atmosphere can interfere with the mass of some atoms. This type of interference is important for m/z values below those of about 82. Thus these interferences are not very important in Pb determination by ICP-MS. The potential interferents are $^{40}Ar^{2+}$, $^{40}ArH^{+}$, $^{16}O_2{}^{+}$, $H_2{}^{16}O^{+}$, $^{16}OH^{+}$, $^{14}N^{+}$, etc. These interferences can be corrected with a blank or by using a different analyte isotope.

Oxide and hydroxide interferences are the most important interferences in ICP-MS. They can be formed from the analyte itself, the matrix components, the solvent and the plasma gases. The more serious interferences are those produced from the oxides and hydroxides of the analyte and of the matrix components. The species formed are MO^{+} and MOH^{+} ions, and these species can overlap the peak of one of the analyte ions. The formation of these species depends on experimental variables such as injector flow rate, radio-frequency power, skimmer conditions, plasma gas composition, solvent removal efficiencies, etc. By taking care with these variables, it is possible to control these types of interference.

Finally, matrix effects are important for concentrations of the interfering element at 500-1000 μg/mL. In general, these interferences produce a reduction in the analyte signal, although in some cases an enhancement is observed. These matrix effects can be controlled by using more diluted solutions, changing the sample introduction procedure, separating the interfering species or using an appropriate internal standard.

3.4.3. Lead determination by ICP-MS

Lead has four naturally occurring isotopes, ^{204}Pb 1.4 %, ^{206}Pb 24.1%, ^{207}Pb 22.1% and ^{208}Pb 52.4%; three of which are radiogenic decay products of either uranium or thorium. The fourth, ^{204}Pb, has a very long half-life and may be considered "stable" on a geological time scale. For this reason, in geological

applications, Pb isotopes are frequently rationed to ^{204}Pb, but unfortunately, the ^{204}Pb isotope is the least abundant and frequently shows the poorest precision of measurement by ICP-MS. The most abundant isotope, ^{208}Pb, is normally the isotope studied.

The detection limit obtained by ICP-MS in lead determination is about 0.1 μg/L; this limit is closest to the limit obtained with Electrothermal Atomic Absorption Spectroscopy and is much better than the detection limit obtained with ICP-AES.

ICP-MS has been used to determine lead in geological materials, waters, food-stuffs, petroleum products and biological materials.

3.5. Thermal ionization mass spectrometry (TIMS)

Thermal ionization mass spectrometry (TIMS) is a mass spectrometry technique in which an isotopic solid material is thermally ionized in a solid-source mass spectrometer. The ions are accelerated into a mass analyzer and then transported to the detector. In general, TIMS uses the magnetic sectors as mass analyzer but thermal ionization quadrupole instruments have also been developed. The most important advantages of TIMS are high sensitivity and high precision. This technique has been used for many years for lead analysis, but in many cases the use of isotope dilution analysis (ID) is necessary. This is because lead ionization depends on many variables such as temperature, time, the chemical form of the sample, etc, and these conditions are not easily reproduced. With the use of ID-TIMS it is possible to obtain a precision of the order of 0.05%.

The combination of the isotope dilution technique and mass spectrometry is considered as a fundamental reference method. ID can be used with TIMS or with ICP-MS, the combination ID-TIMS being normally more precise and more sensitive.

ID-TIMS has been accepted as one of the few definitive methods capable of delivering proven and demonstrated accuracy during the certification process of a primary reference material [69-71].

ID-TIMS is a relatively complex method because the chemical manipulations are done on a mass basis; the mass spectrometer measures isotope ratios rather than absolute isotope intensities. This method eliminates problems such as incomplete recoveries obtained during the sample preparation.

Lead is an element that can be determined using the ID technique because it presents a considerable variation in the isotopic abundance of its four isotopes. To perform an analysis a first aliquot of the specimen is measured for its isotopic composition. A second aliquot is then spiked with an enriched isotope of lead, generally ^{206}Pb and the appropriate isotopic ratio is measured. With this technique it is possible to determine picogram amounts of lead [72]. Nevertheless the high cost of the instrumentation and the need for a specialized

clean laboratory for the chemical separation of lead and the low sample throughput mean that TIMS will remain a research tool for lead measurements.

ID-ICP-MS has been used for accurate determinations of lead in blood [73]; in this method blood is digested with nitric acid in a microwave oven.

In isotopic dilution analysis, a known amount of a rare isotope of lead is spiked in the sample, and the spike is treated as if it were an internal standard. The method has also been used to measure lead in human plasma where the values are very low [74]. To avoid matrix interferences the sample is vaporized in a graphite furnace to separate the lead from the matrix. The detection limit obtained was 3 pg/L.

3.6. X-Ray fluorescence spectrometry (XRF)

3.6.1. General aspects

X-ray fluorescence spectrometry (XRF) is an analytical technique for the elemental analysis of solid and liquid samples with minimal sample pretreatment. The sample is irradiated with X-rays and their absorption produces electronically excited electrons which return to their ground state by transitions involving electrons from higher energy levels. An excited ion is produced when it absorbs radiation, and after a brief period, the ion returns to its ground state via a series of electronic transitions characterized by the emission of X-radiation (fluorescence) of wavelengths identical to those that result from excitation produced by electron bombardment. The energy (or wavelength) of these characteristic X-rays is different for each element and the number of characteristic X-rays of a certain element is proportional to its concentration. The determination of minor and major element concentration (% range) and the trace element analysis (ppm or ppb range) can be performed on the same sample. A particular advantage of XRF is that it is, in contrast to most other elemental analysis techniques, non-destructive of the sample. Depending on how the characteristic X-rays are measured, it is possible to distinguish between wavelength-dispersive and energy-dispersive X-ray fluorescence spectrometry.

3.6.2. Instrument components

An instrument for XRF comprises the five components of instruments for optical spectroscopic measurement: a source, a device for restricting the wavelength range of incident radiation, a sample holder, a radiation detector or transducer, and a signal processor and readout. These components differ considerably in detail from their optical counterparts. It is possible to distinguish between X-ray photometers and spectrophotometers, the first using filters and the second using monochromators to select radiation from the source. In addition, a third method is available: isolation is achieved electronically with devices that have the power to discriminate between various parts of a spectrum based on the energy rather than the wavelength of the radiation. Thus, X-ray

instruments are often described as wavelength dispersive instruments or energy dispersive instruments.

3.6.2.1. Sources

The most common source of X-rays for analytical work is the X-ray tube (Coolidge tube) [13]. This tube consists of a tungsten filament (the cathode) and an anode mounted in a highly evacuated glass tube. The anode is made from a very pure metal such as Cr, Mo, Rd, Ag or W. The cathode is heated by a current from a low voltage power supply. This causes thermoionic emission of electrons from the W-wire. When the negative high-voltage is applied to the filament, the electrons will be accelerated to the anode, which is at ground potential, and bombarded with high energy. The X-rays generated escape from the tube via a beryllium window. Some radioactive substances can be used as sources. The activity of the radioisotopic source is expressed in Becquerels (1 Bq = a disintegration per second). The activity of the source decreases with time. After a time equal to the half-life, the intensity of the source is reduced to 50% of its initial value. The most common radioactive sources are ^{55}Fe, ^{109}Cd, ^{57}Co, ^{125}I, ^{210}Pb, ^{241}Am, ^{147}Pm-Al, The ^{109}Cd are suited to determine the elements from calcium to zirconium.

3.6.2.2. Monochromators

To select the desirable wavelength the use of filters or monochromators is possible [13]. Filters select a part of the radiation and are normally used in X-ray diffraction studies. The choice of wavelengths available with this technique is limited by the relatively small number of target-filter combinations that are available. Figure 16 shows the monochromator of a XRF spectrometer. This consists of a pair of beam collimators, which serve the same purpose as the slits in an optical instrument, and a dispersing element. The dispersing element is a single crystal mounted on a goniometer that permits variation and precise determination of the angle θ between the crystal face and the collimated incident beam. The exit beam collimator and the detector are mounted on a second table that rotates at twice the rate of the first. If the crystal rotates an angle θ, the detector rotates an angle 2θ. The crystals more often used are topaz, LiF, NaCl, ethylenediamine d-tartrate and ammonium dihydrogen phosphate. The most important X-ray lines lie in the region 0.1 to 10 angstrom. However no single crystal satisfactorily disperses radiation over this entire range; for this an X-ray monochromator must be provided with at least two interchangeable crystals.

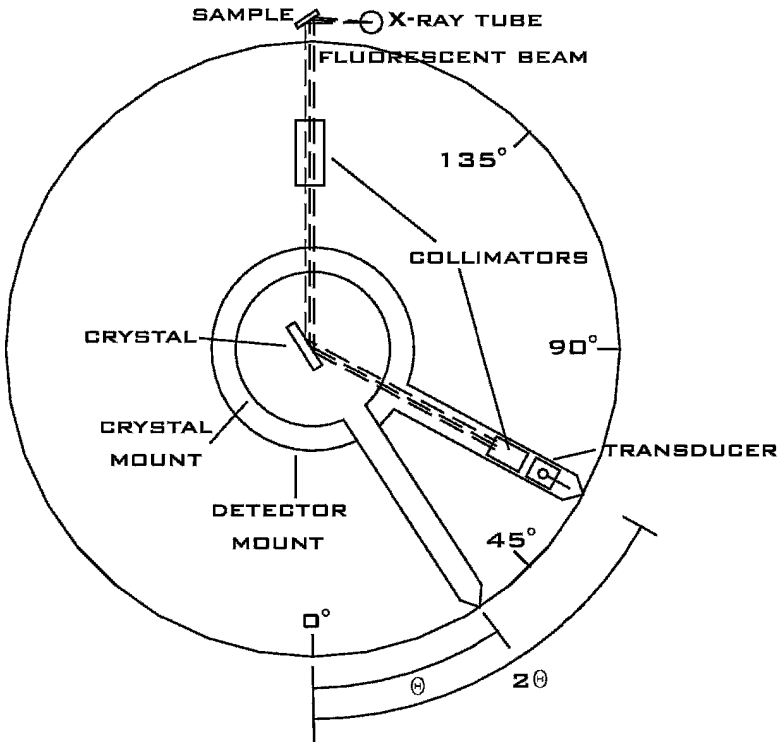


Figure 16. Wavelength dispersive X-ray fluorescence spectrometer.

3.6.2.3. Detectors

The first X-ray instruments employed photographic emulsions for detection and measurement of radiation. However, modern instruments are equipped with transducers that convert radiant energy into an electrical signal. There are three types of detector [13]: gas-filled transducers, scintillation counters and semiconductor transducers. In these transducers individual pulses of charge produced as quanta of radiation are absorbed by the transducer and are counted; the power of the beam is then recorded digitally as the number of counts per unit of time.

Gas-filled transducers. In a gas-filled transducer the radiation enters a chamber through a transparent window of mica, beryllium, aluminum or Mylar. Each photon of X-ray may interact with an argon atom, causing it to lose one of its outer electrons. This photoelectron has a large kinetic energy, and loses this excess kinetic energy by ionizing several hundred additional atoms of the gas. Under the influence of an applied potential, the mobile electrons migrate toward the central wire anode while the slower moving cations are attracted toward the

cylindrical metal cathode. Depending of the applied potential, the number of electrons that reach the anode are different and three different detection systems are possible: (1) the ionization chamber, when the number of electrons reaching the anode is constant and represent the total number formed by a single photon; (2) the proportional counter, where the number of electrons increase rapidly with applied potential, due to a secondary ion-pair production caused by collisions between the accelerated electrons and gas molecules; (3) the Geiger detector, where the amplification of the electrical signal is enormous but is limited by the positive space charge created as the faster moving electrons migrate away from the slower positive ions. The number of electrons reaching the anode is independent of the type and energy of incoming radiation and is governed by the geometry and gas pressure in the tube.

Scintillation counters. A scintillation detector consists of a transparent crystal of NaI activated with 0.2% TlI. Organic scintillators such as stilbene, anthracene and terphenyl can also be used. The incoming radiation traverses the crystal and its energy is first lost to the scintillator; subsequently this energy is released in the form of photons of fluorescence radiation. Thousand of photons are produced by each primary photon over a period of about 0.25 microseconds which is the dead time. The flashes of light produced in the crystal are transmitted to the photocathode of a photomultiplier tube and are, in turn, converted to electrical pulses which can be amplified and counted. The number of photons produced is proportional to the energy of the incoming radiation.

Semiconductor transducers. Today, these detectors are the most important for X-ray. One example of these detectors is the lithium-drifted silicon detector, Si(Li). In this detector there are three layers in the crystal: a p-type semiconducting layer that faces the X-ray source, a central intrinsic zone, and an n-type layer. A negative voltage is applied to the crystal. When an X-ray enters the detector, its energy is absorbed by the crystal. This produces electron-hole pairs. Electrons are promoted from the valence to the conduction band, leaving positive holes in the valence band. Thus the crystal becomes temporarily conducting. Because of the applied bias voltages the electrons are swept to the rear contact, and the holes to the front contact, and for a very short moment of time a current will flow through the crystal. This current is proportional to the energy of the X-ray that entered the detector. In contrast to the flow-proportional and the scintillation counter, the semiconductor detector has no internal amplification. To compensate for this, a very sensitive preamplifier is used to convert the charge to a voltage pulse.

3.6.2.4. Signal processors

All instruments used to measure X-ray radiation are equipped with discriminators which reject pulses of about 0.5 V or less; the transducer and amplifier noise is then reduced significantly [13]. The discriminator can be substituted by pulse-height selectors. These are electronic circuits which reject

not only pulses with heights below some predetermined minimum level, but also those above a present maximum level; they remove all pulses except those that lie within a limited channel or window of pulse heights. Dispersive X-ray spectrometers are equipped with pulse-height selectors to reject noise and to supplement the monochromator in separating the analyte line from higher order. To obtain the energy spectra, pulse-height analyzers are used. These consist of one or more pulse-height selectors. Multichannel analyzers contain up to a few thousand separate channels, each of which acts as a single channel that corresponds to a different voltage window. The signal from each channel is then accumulated in a memory location of the analyzer corresponding to the energy of the channel, thus permitting simultaneous counting and recording of an entire spectrum.

3.6.3. X-ray fluorescence spectrometers

X-ray fluorescence spectrometers can be of two types: wavelength dispersive and energy dispersive.

3.6.3.1. Wavelength dispersive spectrometers

These instruments always use an X-ray tube as the source due to the losses suffered in the monochromator. There are two types of instrument: the single channel or sequential and the multichannel or simultaneous. The spectrometer shown in Figure 16 is a sequential instrument with a single channel. Single channel instruments can be manual or automatic. The manual ones are adequate for the quantitative determination of few elements, whereas the automatic are more adequate for qualitative analysis, where an entire spectrum must be scanned. Actually, spectrometers are provided with two X-ray sources, one for longer wavelengths and the other for shorter. Moreover, for wavelengths longer than 2 Angstroms it is necessary to remove air between the source and the detector by pumping or using a helium flow. In the multichannel dispersive instruments, the simultaneous determination of more than 20 elements is possible. The individual channel has an appropriate crystal and a detector which are arranged radially around an X-ray source and sample holder. Each transducer is provided with its own amplifier, pulse-height selector and integrator. A multielemental determination can be completed in a few seconds. Samples in the form of metals, powdered solids, evaporated films, pure liquids or solutions can be used.

3.6.3.2. Energy dispersive spectrometers

Figure 17 shows an energy dispersive spectrometer that consist of a polychromatic source (an X-ray tube or a radioactive material), a sample holder, a semiconductor detector and the different electronic components required for energy discrimination. The most important advantage of this spectrometer is its simplicity, i.e. without collimators and crystal diffractors. This, and the

closeness of the detector to the sample, results in a 100-fold increase in energy reaching the detector, thus permitting the use of weaker sources, which are cheaper and less likely to cause radiation damage to the sample. In a multichannel spectrometer all of the emitted X-ray lines are measured simultaneously. The principal disadvantage of this instrument is its low resolution at wavelengths longer than 1 Angstrom.

3.6.4. Advantages and limitations of X-ray fluorescence methods

X-ray fluorescence has some advantages. The X-ray spectra are relatively simple, with few lines, so the spectral line interferences are not very important. The technique is not destructive and can be used to study valuable objects without harm or can be used for *in vivo* studies. It is possible to study very small samples and massive objects. Another advantage is the speed of the analysis, making a simultaneous determination possible in a few minutes. The accuracy and precision of the X-ray fluorescence method is similar to other methods.

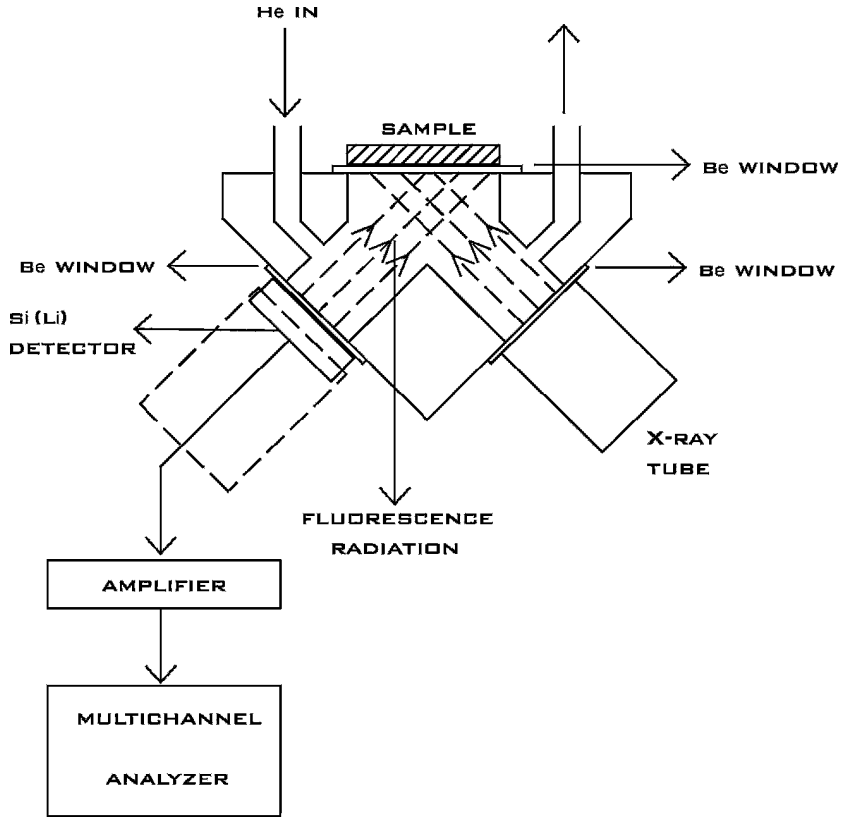


Figure 17. Energy dispersive X-ray fluorescence spectrometer.

X-ray fluorescence methods do have limitations. They are not as sensitive as the various optical methods studied previously. Concentrations of a few ppm can be measured, but in general the concentration range of the method is between 0.01 and 100%. The methods for the lighter elements are inconvenient; difficulties in detection and measurement become progressively worse as atomic numbers become smaller than 23, due to a competing process called Auger emission which reduces the intensity of fluorescence. Another limitation is the high cost of the instrumentation, principally for automated and computerized wavelength dispersive systems.

3.7. Anodic stripping voltammetry (ASV)

3.7.1. General aspects

Anodic stripping voltammetry (ASV) is the electroanalytical technique that has the lowest limits of detection for metal determination with a range of ppb and below. This technique is based on the preconcentration of the analyte present in a solution of a small volume or in the surface of an electrode and the subsequent stripping from the electrode by the use of a voltamperometric technique. If the experimental conditions during the preconcentration step are constant, using adequate calibrations, the measured voltamperometric answer parameter (for example, the peak area) can be used to determine the concentration. The most important advantage of this technique in comparison with direct voltamperometric analysis is the preconcentration of the analyte on the electrode: preconcentration factors between 100 and 1000 can be obtained.

Conventional electrochemical cells with three electrodes are the most often used, although today it is possible to use microcells to work with small sample volumes (0.01-1 mL). Figure 18 shows a typical electrochemical cell for ASV. In the cell there are three electrodes, a reference electrode, usually a calomel electrode, a counter electrode, which is often a coil of platinum wire or a pool of mercury that simply serves to conduct electricity from the signal through the solution to the microelectrode, and the working electrode. This working electrode must have good reproducibility on its surface and show little background current. There are two types of working electrode: mercury electrodes and solid electrodes, the most common being mercury. Among mercury electrodes the most often used is the hanging mercury drop electrode (HMDE). This consists of a very fine capillary tube connected to a mercury-containing reservoir.

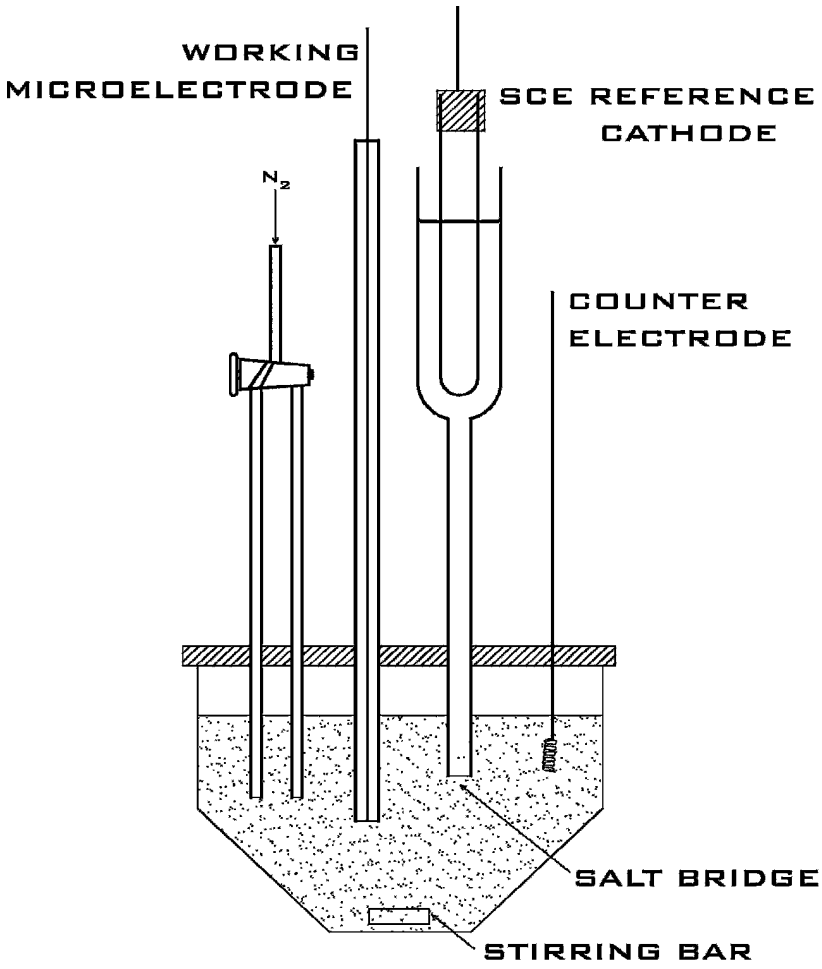


Fig. 18. A typical cell for ASV.

The metal is forced out of the capillary by a piston arrangement driven by a micrometer screw. The micrometer permits formation of drops which have surface areas that are reproducible to 5% or better. After the stripping step, the drop falls and a new drop is produced. This electrode presents a low surface/volume ratio and, as such, the efficiency of the electrodeposition step is reduced. Another type is the static mercury drop electrode (SMDE), in which the drop is formed very quickly by application of an electronic signal and the drop remains until a new electrical signal. Both processes of formation and of the fall are perfectly controlled. With this electrode it is possible to obtain more reproducible and stable drops. The mercury film electrode (MFE) is only used in stripping work and consists of a very thin layer of mercury (less than 100

Angstrom) that covers an electrodic support. The support of the mercury film must have a high electrical conductivity, must be electrochemically inert and must not react with mercury and with the sample components. The most common support used is carbon (in the form of vitrified carbon), pyrolytic graphite, and graphite bonded to epoxy resins, etc. The selection of the type of electrode of hanging drop or the field depends on the analytical problem to be solved.

ASV is used to determine metallic ions by a cathodic electrodeposition and a posterior anodic stripping with an appropriate variation of potential. Figure 19 shows a scheme of the process. The electrodeposition step is carried out in a stirred solution with a constant potential, E_d, to permit the reduction of metallic ions to form the corresponding amalgam. This potential depends on the metal to be studied, and it is maintained during a period of time, t_d, which depends on the analyte concentration. During this step the current is constant, id, and the number of mol deposited is $i_d \cdot t_d/nF$. The second step is a period of rest, t_r, (step b in the figure) during which the stirring is stopped and the applied potential is the same, E_d. This is to permit the solution to rest and so that the metal concentration in the amalgam will be uniform. Afterwards, the step of stripping (step c in the figure) is carried out varying the potential up to positive values. The intensity of the voltamperometry answer, i_p, is the experimental parameter that is related to the analyte concentrations.

The conditions for electrodeposition must be carefully controlled and must maintain constants for the standards and for the sample. It is necessary to control the deposition potential, the deposition time, the speed of stirring and the size of the drop if HMDE is used.

The potential of deposition is selected according to the metal. For deposition time, a longer time period produces a greater amount of the analyte for the stripping step, but very long times can produce undesirable phenomena such as the formation of intermetallic species, the saturation of the mercury, etc. In general for a concentration of analyte between 10^{-7} and 10^{-8} M, times of 2-12 minutes are sufficient. Another important aspect is the speed of stirring. An increase in the speed of stirring produces a greater concentration gradient in the surface of the electrode and, as a consequence, an increase in the intensity, i_d, and an increased amount of the metal deposited.

During the deposition step the analyte concentration in the electrode surface shows a parabolic distribution, which is more uniform if the deposition time is increased. However, the stripping step needs a uniform distribution of the concentration in the electrode. To this end, after the deposition step, the stirring is stopped, then the current decreases and a uniform distribution is achieved (rest step). In general, for HMDE this period can be 30 s or 2 s for film electrodes.

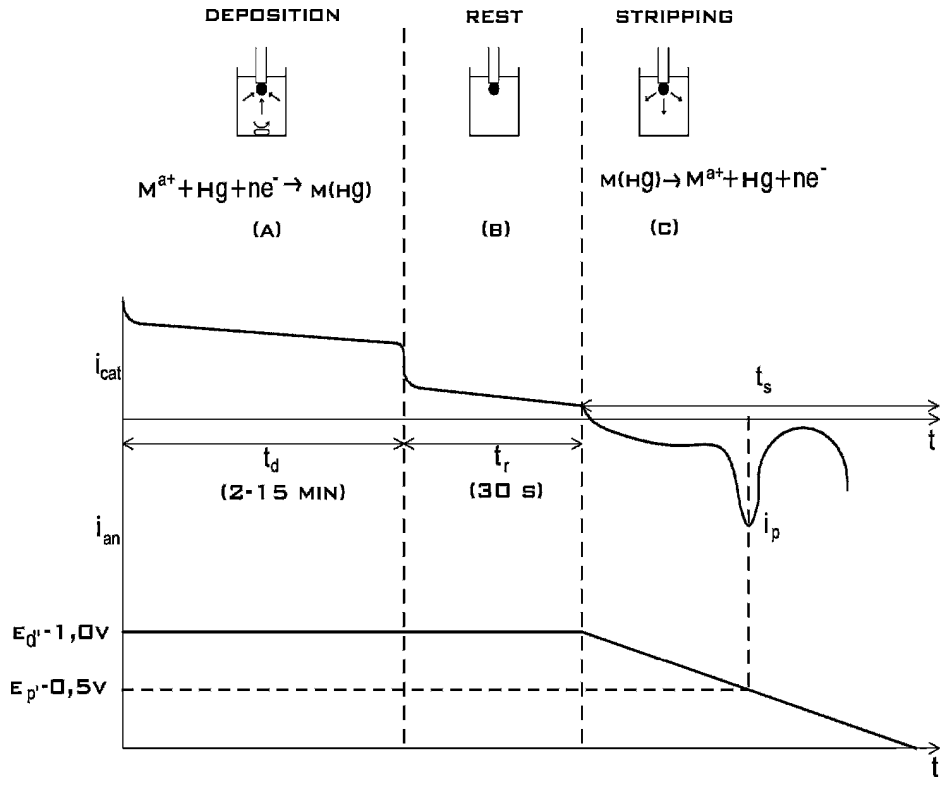


Figure 19. Foundations of ASV.

In the stripping step the scan of the potential is adjust towards more positive values. When the potential of the corresponding redox pair is obtained, electrochemical oxidation is produced which gives the stripping peak, which is proportional to metal concentration. For HMDE the equation for intensity for a linear scan voltammetry is:

$$i_p = AD_M^{1/2}C_M^*\left[\left(2{,}69.10^5\right)n^{3/2}v^{1/2} - \frac{\left(0{,}725.10^5\right)nD_M^{1/2}}{ro}\right]$$

where i_p is in amperes, A is the area of electrode in cm^2, D_M is the diffusion coefficient of the metal in the amalgam in $cm^2\ s^{-1}$, C_M^* is the concentration of the reduced form, M, in the mercury drop, mol cm^{-3}), v (speed of scan, v s^{-1}) and ro in cm.

This equation is valid for scan speeds greater than 20 mvs^{-1}; for very low scan speeds it is necessary to introduce some correction terms into the equation.

With the use of mercury electrodes, the existence of interferences due to the reactions of the metals with the mercury or at the formation of intermetallic compounds between two metals deposited simultaneously on the mercury are possible (for example Cu-Cd or Cu-Ni). These effects are more important with field electrodes than with drop electrodes, because the drops permits the formation of very concentrated amalgams. This can be a factor when choose between MFE or HMDE.

3.7.2. Lead determination in blood by ASV

ASV has been used for lead determination in biological samples, principally for lead determination in blood. A commercially available ASV instrument for blood is used [75]. The electrode assembly includes the test or working electrode, made of carbon, with a thin mercury-film surface, a mixing device, a reference electrode (silver-silver chloride or saturated calomel electrode) , and a counter electrode (platinum or platinum alloy) to carry most of the current. An electronic unit is used to control the potential of the electrodes, initiate and complete the plating cycle, integrate the current time curve, and calculate the analytical results, which are normally expressed in μg/dL [76].

To use this technique, the blood samples must be pretreated prior to analysis to convert lead into free Pb^{2+} for subsequent plating onto the working electrode. This is achieved by digestion in mineral acids or by incubation in a decomplexing reagent. Mixtures of sulphuric acid-perchloric acid or sulphuric-nitric-perchloric acids [78] have been proposed to mineralize the blood sample, but the method recommended by the manufacturer of the most widely used commercial ASV instrument for lead determination in blood is incubation with a decomplexing reagent [76]. This reagent is used to decomplex the lead from erythrocytes or complexing agents (EDTA). The more common decomplexation reagents contain the following ingredients: chromium chloride hexahydrate, calcium acetate monohydrate, mercury ion and Triton X-100; these components must be high-purity components to minimize lead contamination to levels below detection limits of the methods. The sample incubation times in decomplexing reagents vary from as little as 45 min to 18 hour (overnight).

Commercial ASV instrumentation is reported to be linear up to 100μg/dL. A possible problem is the analysis of high-concentration samples because this can produce memory or carry-over effects, so running a blank sample following a high sample is desirable. On the other hand, it may be necessary to run two or more commercial controls to verify the calibration curve; normally, if it is not within limits (about ± 10%) the instrument should be recalibrated.

Copper can interfere with blood lead determination by ASV. When lead and copper strip from mercury amalgams into a supporting electrolyte to the +2

oxidation state, the lead and copper peaks are well resolved if one uses staircases of differential pulse techniques. However when the supporting electrolyte has a high enough chloride ion concentration, copper will strip to the +1 oxidation state and the resulting peak will be much broader. This can produce loss of peak resolution and then false negative blood lead results can be obtained. This interference can be minimized by careful selection of the integration set point,the potential at which current-time area integration is initiated.

At low blood levels ETAAS are probably superior to the ASV technique, although both are useful at higher levels. ASV offers the additional possibility of simultaneous measurement of concentrations of cadmium and other metals.

4. LEAD DETERMINATION IN BIOLOGICAL SAMPLES

4.1. General aspects

The different analytical techniques studied previously have been used for lead determination in biological samples; normally the technique used depends on the type of sample. For some samples, such as blood or urine, direct determination is possible, whereas for tissues, hair, etc., a preliminary step of sample preparation to convert the solid sample in a solution is always necessary.

Due to the low levels normally present in biological samples, a few μg/L or μg/Kg, some precautions are necessary to avoid contamination. Owing to ubiquitous lead pollution, extreme caution must be taken to avoid contamination of biological specimens. Almost every step from sample collection to final lead detection is susceptible to the introduction of exogenous lead. Dust may contain more than 1% lead and a few particles can increase the lead content considerably. Moreover, hair and skin cells shed by laboratory personnel often contain much higher concentrations than the samples being analyzed. To avoid these contamination problems, all materials used must be cleaned with diluted nitric acid at least 24 hours before use, and all the reagents must be of ultra-pure quality. Blank samples should always be incorporated in each batch. In special cases, procedures have to be carried out in a laminar flow or in a clean room.

4.2. Blood samples

Lead determination in blood can be performed by FAAS but a prior step of sample preparation and an extraction step to preconcentrate the sample is necessary. Thus, extraction with HCl, H_3PO_4 and MIBK [79] and with 4-methyl-2-pentanone [80] has been proposed. The results obtained by both procedures agreed with those determined by ASV.

Lead determination in blood samples impregnated on Schleicher and Schull filter paper No.903 (SS-903) has been carried out using the Delves Cup technique [81]. To increase the sensitivity of the FAAS, the use of slotted quartz

tube atom trapping has been proposed [82]. The blood sample was ashed in a Teflon microbomb with nitric acid 2:1 and the solution was directly injected from the microbomb into the flame; a detection limit of 0.33 μmol/L was obtained. The use of flow injection, a nebulizer interface and a computer signal evaluation system [83] has been proposed in order to increase the flame sensitivity. The system improves the detection limits 12-fold compared with those obtained by unmodified instrumentation (limit of detection of 0.06 μmol/L).

The ETAAS is perhaps the most important method for the determination of lead in blood. Some approaches have been proposed for using this technique: (a) direct introduction of the sample into the furnace; (b) dilution with water, Triton X-100 or acid; (c) deproteinization with nitric acid; (d) matrix modification; and (e) solvent extraction.

Direct injection is the least popular because it presents a number of problems:

- It is difficult to pipette microliter volumes of blood accurately due to the wetting of the pipette tips [84,85] and due to the settling of red cells [86].
- The blood becomes soaked into the tube and produces unacceptable memory effects with subsequent analysis [84,86].
- The injection of blood results in foaming, frothing and fogging of the quartz window [87].
- Poor contact of the sample with the furnace walls results in loss of sample out of the furnace ends during the ashing step [88].
- A non-volatile carbonaceous residue build-up occurs within the furnace after only a few injections [84-85, 87-89].
- The background absorption often exceeds 0.5 absorbance units, masking the lead signal and going beyond the compensating capabilities of the continuum background correctors, such as the deuterium lamp [88,90].

To avoid the problems of direct introduction some authors proposed dilution with water. Dilution factors of 5, 10, 20 and 50 were employed. However, even with a 10-fold dilution there is considerable carbonaceous residue build-up and consequent loss of sensitivity [91]. At 10-fold dilution the peak height signal of lead was suppressed by about 20% in whole blood relative to aqueous lead standards [92], suggesting the presence of vapor-phase or chemical interference. At 20-fold dilution, there was apparently no residue, but the ashing step was critical [93]; at the temperature required for complete pyrolysis ($\geq 400°C$), there was loss of lead and at temperatures $\leq 400°C$ the pyrolysis was incomplete, resulting in some production during atomization. Another problem is the background signal that could not be accurately compensated for using the deuterium lamp [94]. The background absorption was brought below the compensating capabilities of the BG by a 50-fold dilution [95]. The depression of the peak height absorbance signal of lead in blood relative to the aqueous lead

standard was caused by the chemical interference of inorganic matrix salts in the vapor phase [96]. At the atomization temperature used, both the lead and the matrix species could coexist in the vapor phase and the conversion of lead to $PbCl_2$ is thermodynamically favorable and this could cause a decrease in the peak height absorbance signal of lead in blood in comparison with an interferent-free solution of the same lead concentration.

To overcome the matrix interference problems, the use of probe atomization has been proposed [96,97]. Blood samples diluted five-fold with water were placed in the graphite probe; the sample was dried and ashed outside the atomizer, then the ashed sample was introduced into the graphite tube which had been preheated to the atomization temperature of 1900°C. The rapid atomization of the ashed sample reduced the vapor-phase interferences and permitted calibration with aqueous standards.

To avoid problems of water dilution, dilution with the surfactant Triton X-100 is preferable. This agent causes complete lysis of the red cells, minimizes frothing, reduces the sample/graphite interfacial tension, improves the contact between the sample and furnace wall or platform, exerts no chemical effects on samples and standards and provides a clear solution [88] . Different dilutions between 1:1 and 1:20 with Triton X-100 with concentrations between 0.005 and 3.4% have been used [98,99]. The Triton X-100 concentration must be carefully optimized to provide little foaming action, but sufficient lytic power to disrupt the cell; the optimum concentration is between 0.5 and 2 % [88]. Even at the optimum Triton X-100 concentration, the build-up of carbonaceous residue is a problem.

Deproteinization of the blood with nitric acid followed by injection of the supernatant into the atomizer eliminated the problem of carbonaceous residue and minimized non-atomic absorption. With the mixture 1:1 blood/nitric acid or 1:1:1 blood/nitric acid/water, the background signal constituted only 7-8% of the signal obtained from whole blood samples; in contrast, the dilution 1:2 Triton X-100/sample produced a background signal which was 31.4% of the total signal [100]. This background signal is due to the molecular absorption and scattering by the chloride salts of Na, K, Ca and Mg forming the inorganic concomitants of the blood matrix. These compounds exhibit strong molecular bands at both 217.0 and 283.3 nm lead line. Nevertheless, the corresponding nitrates absorb only weakly in the spectral region of interest. With nitric acid there is a conversion to nitrates and a reduction in the background signals [101,102]. On the other hand, the addition of concentrated nitric acid to blood constitutes a wet-ashing procedure and destroys the organic matter, minimizing the formation of carbonaceous residue and the smoke produced. The use of concentrated nitric acid, however, caused severe oxidation of the pyrolytic coating of the graphite tube, shortened tube life, degraded precision and sensitivity and increased the risk of contamination. To avoid these problems some authors [103-105]

deproteinized the blood with 6.25% nitric acid and analyzed the supernatant. Under these conditions the use of the pyrolysis step is not necessary [106], which shortens the analysis time. But with the use of nitric acid, the inorganic salts could not be completely removed at the optimum permissible pyrolysis temperature of ≤ 600°C. The use of standard addition is necessary to obtain accurate and precise results.

In order to decrease the background absorption signal, the use of a higher pyrolysis temperature is desirable. In this case, the use of matrix modification that allows lead stabilization at higher pyrolysis temperatures was proposed. Different chemical modifiers have been proposed for lead determination in blood samples, but the most common modifier was phosphate in different forms such as diammonium hydrogenphosphate, ammonium dihydrogenphosphate or phosphoric acid. In the concentration range of 0.2-0.5 % (as phosphate) pyrolysis temperatures of 700-800°C can be used without lead loss [107-123].

This modifier is used together with nitric acid and Triton X-100 at different concentrations. The use of this modifier with the STPF conditions (see Section 3.2.7), principally with the use of the L'Vov platform and the Zeeman Background correction system, decreases the interferences in the direct determination of lead in blood samples. Nevertheless, the use of the standard addition method is necessary in some cases. Phosphate as a chemical modifier was also used in some cases where a treatment of blood sample was carried out. The whole blood sample was diluted with a solution containing Triton X-100, phosphoric and $(NH_4)_6Mo_7O_{24}$ and was incubated with nitric acid at 37°C for 30 min, ashed at 650°C for 20 seconds and atomized at 1800°C for 5 seconds [124]. Other authors [125] have reported that the use of ammonium dihydrogenphosphate destroys the blood sample with nitric acid–hydrogen peroxide (4:1) at 160-180°C and dissolved the final residue with 2% nitric acid.

ICP-MS has been used for lead determination in blood previous to digestion of the blood samples by calcination for 24 hours and dissolving the residue in nitric acid 5 mM. Bi (10 ppb) was used as the internal standard [125]. On the other hand, ICP-MS with isotopic dilution (ID-ICP-MS) has been applied to the certification of lead in four levels of a reference material of NIST blood SRM,955a showing good precision and accuracy [126].

Anodic Stripping Voltammetry (ASV) has been used for lead determination in blood samples (see Section 3.7). A commercial ASV instrument for blood-lead analysis is available [75]. In general, blood samples must be pretreated prior to analysis to convert lead into the free Pb^{2+} state for subsequent plating onto the working electrode. This may be accomplished either by digestion in mineral acid or by incubation in a decomplexing reagent. The acids used were H_2SO_4-HNO_3 and $HClO_4$ [127] or HNO_3 [128]. The most common procedure was incubation with a decomplexing reagent, use $CrCl_3$, $Ca(OAc)_2$, $HgCl_2$ [129] and Triton X-100, and must be prepared with reagents

of high-purity to minimize lead contamination to levels below the method detection limit. Alternative formulations such as the electrolyte of Britton-Robinson [130] have been used, obtaining good results. The incubation time with the decomplexing reagents varies from as little as 45 minutes to 18 hours (overnight). The detection limits obtained with this technique vary from 5 μg/L [128] to 0.1 μg/L when an Au electrode and tetraethylammonium iodide were used [131]. Although the ASV is less used than ETAAS, the results obtained with these techniques are similar in terms of precision, accuracy and sensitivity.

4.3. Urine samples

Lead determination in urine must be carried out by classical methods such as spectrophotometry. The most commonly used reactive was dithizone [132,133], but a previous step of organic matter destruction is necessary; this step is usually performed by an acid digestion using H_2SO_4 and $KMnO_4$ at 37°C for 24 hours. The lead-dithizone complex is extracted in chloroform and measured at 510 nm. Another reagent used is 4-(2-pyridylazo)resorcinol (PAR) [134] in microemulsion medium with SDS-n-BuOH-n-heptane. The absorption maxima of the complex is at 512 nm, and the molar absorptivity was $1.92.10^5$ (Beer's law obeyed at 0-10 μg/25 mL).

The FAAS can be used for lead determination in urine, but the sensitivity is not sufficient and the use of some previous preconcentration procedures is necessary. In this way an exchange reaction involving Ca-EDTA and ammonium pyrrolidine dithiocarbamate has been used; with this reaction it is possible to determine up to 4000μg Pb/L [135]. Another possibility is lead preconcentration by precipitation using $Bi(NO_3)_3$ [136]; this method is simple, accurate, sensitive and rapid. More recently the use of a supported liquid membrane (SLM) methodology was proposed for sample clean-up and enrichment of lead in urine prior to determination by FAAS [137]. Lead ions at pH 3 were extracted across a membrane solution containing 40% di-2-ethylhexylphosphoric acid and dissolved in kerosene, then back-extracted into an acceptor solution of 1 M nitric acid. The mechanism of mass transfer is a proton gradient across the membrane. The enrichment factors were up to 200 with an extraction efficiency of 95%. The detection limits was 6.0 μg/L. The results obtained by this method agreed with those obtained by direct ICP-MS for reference urine samples and samples from occupationally lead exposed workers. Another procedure is the use of a newly developed loop FAAS. The sample to be analyzed is dried outside the flame on an electrical heated Ir loop [138]. The loop is introduced into the flame by a precise transport mechanism. By using an additional ceramic stagnation tube arranged in the flame, the power of detection is increased by about a factor of 10. The detection limits are 3-10 μg/L depending of the AA-spectrometer used.

ETAAS has been used for lead determination in urine samples and has two advantages: sensitivity and the possibility of direct measurement without the need for sample pretreatment. To perform this determination the use of chemical modifier, the L'Vov platform and the Zeeman Background Correction System has been proposed. Modifiers that use phosphate in different forms have also been used for the direct determination of lead in urine. The mixture $NH_4H_2PO_4$-ascorbic acid [139] has been used, obtaining a limit of detection (LOD) of 1 μg/L. A similar LOD can be obtained using H_3PO_4 and the impregnation of the graphite tube with ammonium molybdate solution [140]. Noble metals such as palladium or platinum have been proposed for the direct determination or for ammonium pyrrolidinedithiocarbamate-Me-iso-Buketone extraction [141]. With these modifiers the pyrolysis temperature in both the aqueous solution and the organic extract can be up to 1200°C; the LOD of the direct determination was 0.9 μg/L. Another system used was the automated graphite-probe atomizer [142] which was used for direct analysis of diluted urine samples. The method gives a LOD of 4 μg/L at 283.3 nm and 2 μg/L at 217.0 nm. The results obtained were similar at those obtained with conventional ETAAS.

Isotope dilution gas chromatography-mass spectrometry [143] has been used for lead determination in urine and blood. Lithium bis(trifluoroethyl)dithiocarbamate Pb (FDEDTC) was used as a chelating agent but showed strong memory effects, restricting the range of Pb isotopes ratios that can be measured in unknown samples. To overcome this problem the chelate was derivatized with 4-fluorophenylmagnesium bromide to form $Pb(FC_6H_4)_4$. This method was validated by determining Pb in urine standards from the National Institute of Standards and Technology and reference materials from the New York State Department of Health.

Anodic stripping voltammetry (ASV) has been used for lead determination in urine previous sample digestion with a mixture of $HClO_4$-HNO_3-H_2SO_4 [144] and HBr. The LOD obtained was 5 μg/L. The method is simple and rapid. Nevertheless, direct determination is possible with this technique using a fumed silica [145] which is added to urine prior to the nitrogen purge step; this system completely removed sorption interferences by urinary organic constituents. This method is therefore fast, simple and effective for the accurate determination of lead in undiluted urine without pretreatment.

Another technique that allows direct determination of lead in urine is differential potentiometric stripping analysis (DPSA). In this technique it is necessary to acidify the sample with an acid, usually HCl, to a total concentration equal to 0.2 M [146]. The sample is pre-electrolyzed at –1.2 v for 80 seconds by glassy-carbon electrode coated with mercury, the LOD is 2 μg/L; this technique has been proposed for the routine determination of lead in urine [147]. Its advantages over current methods are low cost and minimal requirement for sample pretreatment, but a limitation of the routine clinical

analysis is the time required for each urine lead determination, i.e. approximately 40 minutes.

Polarography has also been used, but a previous step of organic matter destruction is necessary, which is normally carried out with a mixture of HNO_3, $HClO_4$, HCl [148] or with HNO_3, H_2SO_4 and $HClO_4$ [149]. The LOD obtained can be 4 μg/L. These procedures are simple and useful for routine clinical analysis.

4.4. Tissues samples

Due to low levels, lead determination in tissues can be performed by ETAAS with different chemical modifiers. The most common are the phosphates {$NH_4H_2PO_4$ or $(NH_4)_2HPO_4$} [150]. With these modifiers, pyrolysis temperatures of 1100°C can be used for hair, liver and muscle. The mixture palladium and $NH_4H_2PO_4$ has also been proposed for hair, bone and muscle [151]. On the other hand, Bi(III) nitrate has been used, obtaining better LOD value in comparison with the use of palladium nitrate; moreover, due to the lower atomization temperature used, an extension of the life period of the graphite tube is obtained compared with $Mg(NO_3)_2 - NH_4H_2PO_4$ mixed modifier [152]. The use of coated graphite tubes with different metals is another possibility. In this case, a Mo and La-treated pyrolytically coated graphite tube was employed for the Pb and Cd determination in digested tissues. This modifier reduces chemical interferences and interference from uncompensated background signals [153]. Moreover, Zr, W or Ir has also been used to coat the graphite tubes using the phosphate modifier [154].

The tissue samples must be digested, usually using wet digestion procedures. For this, nitric acid [139] and the mixtures HNO_3-H_2O_2 [154], HNO_3-$HClO_4$ and HNO_3-H_2SO_4 [156] have been used. The mixture HNO_3-$HClO_4$ is the most efficient but in many cases the use of only HNO_3 is sufficient. Tissue solubilization with tetraethylammonium hydroxide has been used for hair, liver and muscle with good results [150]. To determine lead at sub-ppb levels, lead ethylation with $NaBEt_4$ to form $PbEt_4$ and the preconcentration of this compound in the graphite furnace at 300°C has been proposed in an automated method, obtaining a LOD of 12 pg/mL [143].

Direct solid sampling ETAAS has been used to determine lead in muscle tissue [156,157], renal and kidney tissues [158], liver [159] , and epithelial tissues such as hair, nail and skin [160]. In general, with the use of solid sampling it is possible to obtain good results with low analytical errors, the results being comparable to those obtained with acid digestions. Solid sampling has been used for the certification of some reference materials such as bovine liver, bovine teeth and bovine bone [161]. The most important advantage of the use of the solid sampling technique is that the lead determination is quicker, possible contaminations are avoided and the LOD is very low. Non spectral

interferences can be overcome using the STPF conditions and spectral interferences can be avoided with Zeeman effect background correction [162].

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) has been used to determine various elements including lead in human autopsy tissues [162], but in many cases the sensitivity obtained with this technique is not sufficient. The sample preparation must be carried out by extraction, wet digestion or dry ashing [163].

ICP-MS is preferable because it gives better LODs. This technique has been used to determine various elements in different types of tissues: soft tissues [164], thyroid tissues [165], liver and kidney [166], calcified tissues [167], human placenta [168], human fetal tissues [169], central nerve tissues [170], dental tissues [171], etc. In general, the samples have been digested using a wet digestion procedure, except for the dental tissues for which laser ablation has been used. In order to improve the detection limit, the use of hydride generation prior to ICP-MS has been proposed; with these systems a LOD of 0.002 ng/mL [172] or 0.007 μg/L [173] can be obtained. On the other hand, the use of ICP-MS allows isotope determination; thus the ^{206}Pb:^{207}Pb ratio has been determined in human tissues such as blood, teeth and in environmental samples [174].

Differential pulse anodic stripping voltammetry (ASV) has been used in the simultaneous determination of various metals, including lead, in different tissues such as cancer tissue samples [175], animal tissues [176] or human blood and teeth [177]; normally the tissues were digested by wet digestion with HNO_3-$HClO_4$. The fifth order differential stripping voltammetry has also been used for the determination of Cu, Pb, Cd and Zn in human brain tissue, which was digested with HNO_3 and H_2SO_4 and the metals were determined in 0.03 mol/L $HClO_4$ as the supporting solution. The relative standard deviation for lead was 10.5%; the method was valuable and simple [178]. Differential pulse anodic stripping voltammetry was compared with AAS in lead and cadmium determination in animal tissues such as poultry liver, muscle and kidney tissue after dry ashing and dissolution in HNO_3. The results obtained by both techniques showed a high level of concurrence, except in the case of some liver samples [179].

An automatic-continuous method for the simultaneous determination of copper and lead based on flow injection analysis (FIA) and stripping voltammetry (ASV) has been proposed [180] for metal determination in fresh bovine liver samples and certified reference materials from the National Institute for Standards and Technology and the Community Bureau of References. The same authors [181] used a similar procedure for the simultaneous determination of Cu, Pb, Cd and Zn in previously lyophilized biological tissues. The performance of the method was validated by a statistical study using parameters of certification campaign of the CRMs obtaining results within, or close to, the confidence levels of the certification campaign. Potential stripping analysis

(PSA), without chemical destruction or organic matter, was applied to Cu,Pb and Cd determination in various animal tissues [182] .Organic matter destruction is avoided by the use of sodium dodecylsulfate and sodium deoxycholic acid solution in a reflux process with the aid of sonication. The efficiency of this digestion was tested by comparison with total mineralization using the low temperature ashing method. The digestion medium is adapted to PSA measurement by the aid of a mixed (aqueous-organic) electrolyte. The process from the lyophilized sample takes approximately 3-4 hours.

X-ray fluorescence spectroscopy (XRF) has been applied to lead determination in different human tissues. This technique has been used in the study of brain tissues for diffuse neurofibrillary tangles with calcification (DNTC), which is a typical dementia characterized pathologically by diffuse neurofibrillary tangles without senile plaques. This method allows the non-destructive determination of Ca, Fe, Zn and Pb in brain tissues [183]. XRF has also been used to study trace elements in liver, brain and kidney tissues of patients with cirrhosis [184]. Chemometric methods, such as cluster analysis and multivariate three-way principal component analysis, have been applied to the analysis of human tissues by XRF for cancer recognition. In this study, more than 20 elements, including lead, were analyzed [185]. Synchroton radiation X-ray fluorescence (SRXRF) was used to determine the multielement composition of the full-term placenta of Russian women [186]. Energy dispersive X-ray fluorescence spectrometry (EDXRF) has been applied to the study of trace element concentrations in human tissues such as bone, hair, liver and kidney [187]. All samples were analyzed without any chemical treatment; the soft tissues (liver and kidney) were lyophilized and ground in an agate mortar, whereas the hard tissue (bone and hair), after being lyophilized, were dried in an oven for 24 hours at 200°C prior to grinding. XRF has also been used to study the distribution of lead in bone [188], a vivo XRF bone lead analyzer has been constructed to be tested as the first step towards developing a deep bone lead analyzer.

5. LEAD DETERMINATION IN ENVIRONMENTAL SAMPLES

5.1. Lead determination in plants

To perform lead determination in plants, a prior step of sample preparation to dissolve the sample is normally necessary. To this end, different digestion procedures have been used. The ashing of the plants at 450°C and dissolving the ash in 6 M HNO_3 is a procedure proposed by some authors [189-191]. Nevertheless this procedure has two limitations: firstly, it is a long procedure, and secondly, there is the possibility of contamination of the sample in the oven. Another procedure is wet digestion with different acids or mixtures

of acids. Different acid mixtures have been proposed: the mixture HNO_3-HCl in closed PTFE bombs in a microwave [192], the mixture H_2SO_4-HNO_3-$HClO_4$ or the H_2SO_4-HNO_3 mixture and later addition of H_2O_2 [193], or the HNO_3-H_2O_2 mixture also in closed vessels with microwave energy [194-197]. Although the mixture more often used was HNO_3-H_2O_2, some authors studied the effect of H_2O_2 and they concluded that better results can be obtained using only HNO_3 [198]. Other authors propose systems that use the dry and wet digestion procedures, ashing the sample at 550°C and digesting it with a mixture of HNO_3-H_2SO_4 [199]. On the other hand, and in order to dissolve the silicate, the use of HF together with HNO_3-H_2O_2 was also recommended [200-201]. This mixture, in closed vessels with microwave energy was proposed as a digestion system suitable for routine applications. More recently, the use of ultrasound assisted solid-liquid extraction with diluted acid such as HNO_3 and HCl has been proposed as a sample preparation method for plant analysis [202,203]. The precision of this procedure, together with its efficiency, rapidity, low cost and environmental acceptability, makes it a good alternative for the determination of trace metals from plant materials. On the other hand, the use of enzymatic hydrolysis accelerated by means of ultrasonication has been proposed for the simultaneous determination of various metals in seaweeds by ICP-AES [204]. This is a promising method to pretreat solid biological samples for multielement determination in a short time, under safe conditions, with chemical species integrity and without great wastage.

FAAS can be used for the direct determination of Pb in plants, This method has been used in the study of lead levels in some herbal plants for curing cardiovascular diseases [205], for different medicinal plants [206], in the study of lead levels in different parts of plants (fruit, twig and root) [206] and for lead determination in seaweeds such as *laminariale undaria pinnatifide* (wakame) [207]. Nevertheless, the sensitivity obtained is not sufficient. In many cases the use of a previous preconcentration method is necessary. Flow injection systems have been used in different preconcentration methods; the preconcentration from solution after wet digestion was performed using a C18 column as sorbent, [208], ammonium diethyldithiophosphate at pH 1 as a complexing agent and methanol as an eluent. In this system Fe and Cu interfere but this interference was successfully eliminated using oxalic acid and thiourea. The enrichment factor obtained at 30 seconds loading time was 38 and the LOD was 4.4 μg/L. Another preconcentration system used is complexation with potassium Pr xanthante and extraction into MIBK [209]. Lead can be precipitated at pH > 1.0 as fluorides using La as a carrier and lead pyrrolidin-1-dithioformate complex can be extracted into a small volume of chloroform. The direct nebulization of the chloroform extract into an air-acetylene flame gives a sensitive signal intensity; this method has been applied to the determination of lead in botanical certified reference materials, giving good results [210]. To avoid the use of

preconcentration methods, the use of a simple, reagent-free solid sampling method has been proposed [211]. The dry sample is ground to a powder and 0.5 g is suspended in 10 mL of deionized water. Aliquots (20 μL) are pipetted into Ni microsampling cups, which are dried at 110°C and inserted into an air-acetylene flame. Suitable dilutions of the suspension provide a linear range of 0.072-240 μg/g of dry vegetation. The method is simple, accurate, faster than comparable methods, and provides adequate precision for this application. Another important aspect in Pb determination by FAAS is the problem of spectral interferences. Some authors studied these problems in plant leaf digest in three ways: with deuterium background correction, with Smith-Hiefte background correction, and without any background correction. The use of a background correction system is deemed to be necessary. The two systems gave equal results for low Fe concentration (< 200 mg/L), but high Fe concentrations (500-2500 mg/L caused spectral interferences which were overcompensated by the deuterium system [212].

ETAAS has been used for lead determination in different types of plants and biota previous to a sample digestion pretreatment, normally by wet digestion using microwave energy [213-215]. The chemical modifiers most used were phosphate modifiers. An interesting method was the use of a fast program in order to shorter the analysis time. Using these programs it is possible to eliminate some steps. Thus a method was proposed without the ashing step, using a graphite tube with L'Vov platform and $(NH_4)_2HPO_4$ as the chemical modifier [216]; the cycle time was 72 s. The application of the fast programs combined with iridium as a permanent modifier was also proposed [217].The coating of the platform or the tube atomization area with Ir is an efficient means of improving the accuracy and precision. The fast program method can thus be recommended for routine determination in plants. However, the most important advantage of ETAAS is the possibility of using slurries or solid sampling. Slurries have been used by various authors [218-222]; these methods are normally very simple and, moreover, present a very good sensitivity level. With the use of slurries there are no matrix effects [219-222] and, in many cases, calibration with aqueous standards is possible. For slurries, the use of the fast program methodology, in which the drying and ashing steps were replaced by a single modified drying step was used [219], the slurries were prepared in a 20% (vol/vol) ethanol medium using 0.1%$NH_4H_2PO_4$. The suspending medium also contained H_2O_2 to prevent the build-up of carbonaceous residue inside the atomizer. The mixture 1M HNO_3 + 1,5M H_2O_2 or tetramethylammonium hydroxide [223] has been proposed to prepare the slurry. The comparison of different ultrasound-assisted extraction with an ultrasonic bath has been studied [224]; optimum performance of solid-liquid extraction was achieved only with probe sonication. Although the use of the direct solid sampling has the advantage of more sensitivity, it has some problems of reproducibility, because

it requires fine grinding (1μm), whereas slurry sample introduction is more tolerant to particle size effects [221]. The use of solid sampling presents some interference problems, thus at the lead 261.4 nm nonresonance line, overcompensation errors were observed which were caused by cobalt when using a Zeeman effect system and undercorrection due to the $AgCl_{(g)}$ molecules was observed with a deuterium background correction system [225].

ICP-AES has been used in the simultaneous determination of various elements, including lead, in different types of plants. In general, the plant sample was digested by a wet digestion procedure using different acids, nitric acid in a closed microwave system [226], the mixtures nitric/perchloric acids [227,228] and the mixture nitric/hydrofluoric acids in a teflon beaker on a hot plate, dissolving the residue with 1 M nitric solution [229]. A simple automated microdigestion process was developed for the determination of 17 elements in plants (feed, forage and crops); 0.25-1.0 g samples were digested with nitric acid in a 10 mL graduated kimax culture tube on a programmed heating block [230]. The small sample size minimizes reagents used and their subsequent waste disposal. Microwave-assisted extraction with water, EDTA and HCl leaching solutions have been proposed for trace metals determinations in plants [231], HCl was found to be very suitable for quantitative extraction of various elements including lead, and EDTA led to a complete extraction; in both cases the relative standard deviations were below 8%. The introduction of volatile species is another approach in the lead determination by ICP-AES. Continuous flow generation of two different types of volatile lead species PbH_4 ($NaBH_4$ as the reducing agent) or tetraethylead ($NaBEt_4$ as the alkylating agent) was studied as a means of gaseous sample introduction for ICP-AES [232]. Lead determination using $NaBH_4$ in a medium of $K_2Cr_2O_7$ and lactic acid as oxidant/complexing acid system provided a LOD of 2 ng/mL with a RSD of 1.3% at the 50 ng/mL level and good selectivity. $NaBEt_4$ used as reducing agent in the same reaction medium provided a LOD of 1 ng/mL, a RSD of 1.2% at the same concentration level and even better selectivity. The use of slurries has also been proposed [233] using a particle size of < 5 μm obtained by wet grinding with Zr oxide beads in the presence of $LiNO_3$. The atomization efficiency was 95% in a direct current plasma spectrometer. Slurry atomization allows solid samples to be introduced into the plasma with minimum modification and allows calibration with simple aqueous standards. The direct introduction of a solid sample has been carried out using a cuvette made of tungsten, where a mixture of a ground solid sample and powder diammonium hydrogenphosphate was precisely weighed [234]. The cuvette was positioned onto the tungsten-boat furnace incorporating a vaporizer. Tetramethylammonium hydroxide solution was added and then the cuvette was heated and maintained at a wet-digestion temperature to decompose the solid sample. After digestion, the temperature was elevated to generate the analyte vapor for introduction into the plasma. Since the solid

samples were wet-digested before vaporization, the use of aqueous calibration is therefore possible. Possible problems in lead determination by ICP-AES are the matrix effects, signal suppression is observed for the ionic lines and signal enhancement occurred for atomic lines [235]. These effects can be caused by particles and easily ionizable elements into the central channel of the plasma. These matrix effects can be removed by using high generator power and a moderate uptake flow rate of solution into the plasma [235-236].

ICP-MS has also been used in the lead determination in plants. The sample digestion can be performed with HNO_3 [225], with the mixture HNO_3-HF [229-238], or with the mixtures HNO_3-H_2O_2 [239-240], HNO_3-HCl and HNO_3-H_2O_2-HF [241]. In some types of sample such as lichens, only the mixture HNO_3-H_2O_2-HF ensures complete recovery, obtaining a precision better than 3-5% [241]. In the procedure that uses HNO_3-HF [238] the use of boric acid was not necessary, which simplified the procedure and minimized the content of the total dissolved solids in solution for ICP-MS analysis. The calibration was prepared using external standard prepared in undigested reagent blanks with In as an internal standard. When the H_2O_2 was used with HNO_3, the presence of H_2O_2 helped to maintain a higher temperature under the pressure limit and reduced the carbon content in the digestates, but its impurities hampered the ICP-MS analysis for certain elements at low levels [240]. Nitric acid has been used alone in a dynamic ultrasound-assisted extraction with diluted acid as extractant obtaining a repeatability of 0.9% and a reproducibility of 2.8% [241]. Ultrasonic slurry sampling-electrothermal vaporization-inductively coupled plasma-mass spectrometry has been applied to the analysis of plant samples [242]; in this case the standard addition method must be used, and the LOD obtained for different elements were between 7.8 and 8 ng/g with a precision better than 24%. On the other hand, laser ablation has been applied to the solid sample introduction into an ICP-MS for the multi-elemental analysis of several environmental matrices. Lead was determined in two reference materials, Tea Leaves CRM from the the Japanese National Institute of Environmental Studies Certified Reference Material and Milk Powder, CRM A11 from the International Atomic Energy Agency [243]. The comparison with certified values shows fairly good accuracy, and the more important advantage is the rapidity of the analyses without sample preparation. In order to increase sensitivity, lead determination by hydride generation ICP-MS has been proposed [244]. For this the oxalic acid ammonium cerium(IV) nitrate-sodium tetrahydroborate has been used as the reaction matrix. The sensitivity achieved was approximately 7.5 times higher than that obtained using an ultrasonic nebulizer. The LOD was 0.007 μg/L and the precision 0.21% for 1 μg/L.

Lead determination in some types of plant has been carried out using anodic stripping voltammetry (ASV) [245-248]. The level of lead was determined in a plant cell culture, obtaining a LOD of 500 pM in a phosphate

buffer [246]. In general ASV was used for the simultaneous determination of various metals such as Zn, Cd, Pb, Cu, Fe and Mn [246]; the samples were dry-ashed and then dissolved in $HClO_4$ using a working electrode of a long-lasting sessile-drop mercury electrode. The use of the standard addition method was necessary. In general the results obtained with ASV were comparable with the results obtained by AAS.

Potentiometric stripping analysis has been applied to the determination of lead and cadmium in biological samples using a mixture of NaCl and HCl as supporting electrolyte. In the presence of 4% lumaton in methanol, the oxidation potentials were –0.46 V for lead and –0.68 V for cadmium. The samples studied were leaves, needles and hair [249].

Another analytical technique used for lead determination in plants is X-ray fluorescence spectrometry. Energy dispersive X-ray fluorescence [250-252], synchroton radiation X-ray fluorescence [253] and total reflection X-ray spectrometry [254] have been applied for the qualitative and quantitative determination of lead in different types of plants. These techniques have been used to study lead distribution in the different parts of plants or to perform trace element accumulation studies in different environmental problems.

5.2. Lead determination in soils and sediments

Due to lead's very low mobility, appreciable quantities may be found in soils and sediments. Some lead has a natural origin but anthropogenic inputs often give rise to elevated levels, for example, the lead content of soil may be high due to the application of lead arsenate as an insecticide and near roads by atmospheric deposition. Normally the determination of lead in soils and sediments may be used as an indication of atmospheric or water pollution. For non-polluted soils or sediments the lead is bound in the silicate lattices of refractory material and can only be released using hydrofluoric acid; the mixture HNO_3-HF has been used for lead extraction, although the mixture HNO_3-HCl is usually adequate to remove most lead from heavily polluted soils. In recent years, with the use of microwave energy, the sample preparation step has been shortened and microwave aqua regia is the recommended method. In some analytical techniques such as ETAAS the use of solid samples in the form of slurries is possible, avoiding the digestion procedures. In order to ascertain the availability of lead, different extraction methods have been proposed, which will be described later in the text.

Colorimetry has been applied to lead determination in soils. A modified rhodizonate spot test was used for the detection of lead contamination of soils at concentrations of 400-700 μg/g [255]. Extraction is carried out by heating or shaking with nitric acid followed by filtration. The filtrate is adjusted to pH 1.5 and is spotted on filter paper and treated with aqueous Na rhodizonate. Soil extraction at pH < 1 showed rapid bleeding of the pink Pb-rhodizonate spot.

Another reagent used for lead determination in soil was 2,5-dimercapto-1,3,4-thiadiazole (DMTD) [256] which reacts in slightly acidic media to give a greenish-yellow chelate that has an absorption maxima at 375 nm. The reaction is instantaneous and absorbance remains stable for 24 h. Linear calibration graphs were obtained for 0.1-40 μg/mL of Pb.

FAAS has been used for lead determination in soil and sediments but the sensitivity is not sufficient and a preconcentration method must be used. Different reagents have been used. Xanthate-1,10-phenanthroline complex onto microcrystalline naphthalene has been proposed for lead determination obtaining a LOD of 2,5 μg/L, which is 200 times lower than for the direct FAAS methods [257]. The diethyldiethyldithiocarbamate (DDTC) complex was used in different forms; it can be retained in a miniature column of Chromosorb 102 and eluted with ethanol [258]. Moreover, the complex can be used in a flow injection online coprecipitation system with DDTC-Ni(II) [261] or DDTC-Cu(II) [260]. In both systems metal ions were coprecipitated online in nitric acid media and the precipitate was collected in a knotted reactor, after it was dissolved in IBMK and transported directly into the nebulizer burner system of FAAS. Detection limits of 2 μg/L or 32 μg/L, respectively, can be obtained. Diethylammonium-N,N-diethyldithiocarbamate (DDDC) or NH_4^+diethyldithiophophate (DDDA) have been used as a complexing agent using octadecyl functional groups C_{18} bonded to silica gel as sorbent [261]; with this system a LOD of 10μg/Kg can be obtained. A similar LOD can be achieved with the use of the dialkyldithiophosphates as chelating agents using octadecyl functional groups C_{18} bonded to silica gel as sorbent and methanol as eluent [262]. Another solvent extraction system used was the iodide complex from acid extracts of soil samples, giving a LOD of 0.30 μg/g [263]. On the other hand, solid phase extraction (SPE), using an immobilized crown ether as an extractor, was applied to the FI-FAAS determination of Pb(II) obtaining a LOD of 0.08 μg of Pb with a RSD of 4.1% [264]. In order to increase the flame sensitivity, the atom trapping system using the slotted tube atom trap has been used by various authors [265-267], obtaining a sensitivity similar to ETAAS.

To avoid sensitivity problems of the flame, ETAAS has been used for lead determination in soil and sediments using digested soils samples or slurries. Different chemical modifiers have been proposed, the most classical, the mixture of palladium-magnesium nitrate, has been used for aqueous solutions and for slurries [268]. Small amounts of palladium delayed the atomization until furnace conditions were nearly isothermal and the presence of magnesium was required in order to avoid a reduction in the analytical recovery. The use of 0.6 μg Pd + 1.0 μg Mg was suitable. For soil slurries, 0.6 μg Pd alone was effective [269]. It is thought that the organic material in soil aids the reduction of palladium and thus improves its effectiveness in delaying lead vaporization. One advantage of the mixture Mg-Pd for slurries is that it produced similar

absorbance appearance and peak maximum times for the slurries and the aqueous calibration standards [270]. Different permanent modifiers (Rh, Ir, Ru, W-Rh, W-Ru, W-Ir) thermally deposited on the integrated platform of the transversally heated graphite atomizer were employed for lead determination in sediment, sludges and soils [271]. The most important advantage of this modifier is that the life of the graphite tube is increased by about 20%.

Fast programs that omit the pyrolysis step have been used for lead determination in aqua regia digest of soils and sediment [272] and in soil slurries [273]. In both cases, the use of chemical modifiers is not necessary. Normally, the duration of fast programs is about 45 seconds. In the use of slurries it is very important that the soil is finely ground. Soil particle size is important as insufficient grinding leads to poor recovery from large particles [274]. A possible problem is that particles can adhere to the magnetic stirring used to suspend the slurry; to avoid this problem vortex mixing as an alternative system [275] can be used.

ICP-AES has been used for the simultaneous determination of various elements, including lead, in soil and sediment samples. Different digestion procedures have been proposed for dissolving the sample: digestion with aqua regia using microwave energy (MW) [276] is the most common. Other methods use the mixtures HNO_3-HF [277,278] and HF-HCl-HNO_3 [279]; these mixtures are recommended when the silicate content of the soil is high. Recently, ultrasound-assisted extraction with diluted solutions of aqua regia [280] has been applied and the results obtained were similar to those of the microwave digestion method or reflux which is the ISO 11466 standard method. The major advantages compared to the MW and reflux method are the high treatment rate (50 samples simultaneously in 9 minutes) and low reagent usage, the main benefit of which is the low chloride and nitrate concentrations in the extracts. Electrothermal vaporization (ETV) of the soil or sediment samples has been applied for volatile and non-volatile elements. Samples were deposited either directly as a solid or by slurry sampling into a graphite cup which was then positioned in the radiofrequency field and vaporized into a carrier flow of 15% SFe-Ar [281]; signal precision was better with slurry sampling, obtaining recoveries about 80-105%. In another ETV system the sample is transported to the plasma with the addition of toluene vapor to the internal furnace gas and sodium selenite to both solutions standards and solid samples; with this mixture transport losses of analyte were considerably reduced [282]; the sensitivity was approximately 20-fold better for the solid sampling ETV than for the dissolution based pneumatic nebulization sample introduction method. The use of slurries is another interesting approach. Reducing soil particle to < 5 μm by wet grinding with Zr oxide beads in the presence of $LiNO_3$ resulted in slurries with an atomization efficiency of 95% [283]. Slurry atomization allows solid samples to be introduced into the plasma with minimal modification and allows calibration

with aqueous standards. For solid samples, the laser ablation sampling technique has also been used using a Q-switched Nd:YAG laser coupled with a new echelle spectrometer which has a multichannel solid-state detector. The laser pulses were focused onto the solid surface of pressed soil samples to generate an aerosol which was introduced into a flowing Ar stream transported through a tube and then introduced directly into the ICP; the RSD was between 4.9 and 12.7 % for different elements [284]. The hydride generation-ICP-AES (HG-ICP-AES) system has been applied to the speciation of lead. A simple and rapid method to determine the sum of tetraalkyllead compounds (Me_4Pb,Et_4Pb) and their intermediate decomposition products, the trialkyllead (Me_3Pb^+, Et_3Pb^+) and dialkyllead (Me_2Pb^{2+}, Et_2Pb^{2+}) species was proposed [285]. Lead species in solution are transformed into the corresponding hydrides by sodium tetrahydroborate and directly introduced into the ICP torch. The presence of an acid is decisive in obtaining highly different sensitivities for organic and inorganic lead; 2% citric acid suppressed the signal of inorganic lead offering the possibility to determine organolead compounds in the presence of excess amounts of inorganic lead, thus characterizing the method as a screening method.

ICP-MS has also been used for the simultaneous determination of trace elements in soil and sediments after a predigestion of the solid sample normally using mixtures of HNO_3-HCl or HF-HNO_3-HCl [286] and using high pressure digestion vessels [287] with MW energy. The dry ashing procedure with $Mg(NO_3)_2$ has been proposed for the Pb, Cd and Sb determination in soil samples [288]. Newly developed methods involving an online combination of sedimentation field flow fractionation with inductively coupled plasma-high resolution mass spectrometry (SdFFF-ICP-HRMS) has been used to study the distribution of extractable heavy metals in soil that had been treated with sewage sludge contaminated with Cu and Pb [289]. The combination of selective chemical extraction and SdFFF-ICP-HRMS provides a means of determining the distribution of potentially available heavy metals within the colloidal fraction of contaminated soils. Laser ablation ICP-MS was applied to the determination of various metals [290]. The dried soil powder was pressed into a pellet for LA-ICP-MS and Triton X-100 was added to work as the modifier to enhance the ion signals. The standard addition method and isotope dilution method were used; the precision was better than 5% and the LOD was 0.3 ng/g. For LA-ICP-MS the particle size could significantly influence the measurements only if the surface constituents of the particle were ablated. Particle size influences the precision and sensitivity of the measurements, samples with smaller particle sizes yielding higher signals levels and better precision [291]. Ultrasonic slurry sampling-electrothermal vaporization ICP-MS was applied to the determination of volatile elements, Cd, Hg and Pb in soil. Palladium was used as the modifier. The precision was better than 4% and the LOD was

between 5 and 76 ng/g in different samples [292]. The isotope dilution ICP-MS has been used for the certification of Pb and Cd in environmental standard reference materials such as SRM 1944 New York-New Jersey Waterway Sediment, SRMs 2583 and 2584 Trace Element in Indoor Dust, SRMs 2586 and 2587 Trace Element in soil containing lead from paint [293]. One advantage of ICP-MS is that it offers the opportunity to measure stable isotopes; when the isotope proportions differ sufficiently among source materials, isotope ratio provides a means to distinguish the origin of pollutants such as lead [294,295].

ASV has been used to determine the total and available Pb in soil and sediments. For the total determination, MW with a mixture of HNO_3 and $HClO_4$ with and without HF was used. The extraction with acetic acid proposed by the Bureau Communitarie de Reference (BCR) was used to assess availability [296]. The levels of lead in EDTA extracts of soils have also been studied by ASV [297]. In general, stripping analysis was successfully employed for field verification of metal contaminants in solids and sediment at hazardous waste sites. The remarkable sensitivity, portability, low power requirement, and low cost of stripping analysis make it an attractive choice for on-site analysis of selected metals during site characterization and remediation activities [298].

Another electrochemical method used in lead determination in soils has been potentiometry and polarography. Potentiometric striping analysis was applied to sediment and sludges with a precision in the range of 2.4-4.1% [299], and stripping chronopotentiometry has been used for determination in soil extracts and gives a LOD of 10 μg/L for a deposition time of 120 s [300]. Polarography has been used for the simultaneous determination of lead and cadmium in environmental samples after adsorption of their morpholine-4-carbodithioates onto microcrystalline naphthalene or morpholine-4-4-dithiocarbamate-CTMAB-naphthalene adsorbent [301], or after adsorption of their 2-nitroso-1-naphthol-4-sulfonic acid-tetradecylmethyl benzylammonium ion-associated complex on microcrystalline naphthalene [302].The detection limits obtained were 0.4 μg/mL and 0.10 μg/mL of lead, respectively.

An inexpensive, safe, fast and non-destructive method for lead determination in soil and sediments is the X-ray fluorescence (XRF). In the analysis of sediment the calibration can be carried out by adding known quantities of $Pb(NO_3)_{2\,(aq)}$ to known masses of a sediment matrix made from all the samples; this procedure eliminates the need for standard additions [303]. For analysis of soil with high metal content, the samples must be dried and powdered previously, and in these conditions a LOD of 19 ppm Pb can be obtained [304]. For the analysis of low level contaminated soil the use of the $LiBO_2$ and $Li_2B_4O_4$ fusion method and the use of synthetic standard prepared in Al_2O_3 matrices were proposed [305]. To determine the heavy metal content in soil samples at contaminated locations, a static and time-consuming procedure is used in most cases: soil samples are collected and analyzed in the laboratory at

high quality and high analytical cost. There is a growing demand from governments and consultants for a more dynamic approach and from customers who require that analyses are performed in the field with immediate feedback of the analytical results. Therefore, the use of portable instruments is recommended. For over 30 years portable XRF analyzers have been available for the in-situ nondestructive measurements of lead in paint. Recent advances have made their use possible for analysis of airborne dust filter samples, soil and dust wipes [306]. The applied soil pretreatment was found to play an important role in the quality of the XRF data obtained, independent of the type of instrument used. In situ measurements were strongly influenced by the heterogeneity of the analyzed area. A limited pretreatment like sieving < 2 mm significantly improved the XRF data quality [307]. In general the results obtained with the portable instruments are comparable to the results obtained with the high performance laboratory XRF system [308] or with the results obtained using ICPAES [309].

5.3. Lead determination in water

The most important problem in lead determination in water is the low levels present in noncontaminated water (about few μg/L). In many cases, a preconcentration step is necessary before lead determination by the analytical technique. UV-VIS spectroscopy has been used for this determination .The typical method is the dithizone method using a new flow injection system with a preconcentration step on chitosan, obtaining a LOD of 5.0 mg/mL and a sample throughput of 15/hour [310]. 4-(2-pyridylazo)resorcinol has been used after the sorption of lead as its thiosulfate complex on a fibrous sorbent fillet with AV-17; a LOD of 0.01 mg/L has been obtained [311]. The same complexing agent has been used with the reversed flow injection analysis (FIA) technique in a 0.1 M borate buffer and with a preconcentration on a microcolumn of spheron oxin 1000 chelating sorbent, obtaining a sensitivity of 0.01-0.06 μmol/L for a sample volume of 20 mL [312]. A calixarene-based polymeric chelating resin synthesized by covalently linking calix(4)arene-o-vanillinthiosemicarbazone through its lower rim to Merrifield resin. was used to separate and preconcentrate Cu(II), Cd(II) and Pb(II); the LOD for Pb was 19.61 μg/L with a preconcentration factor of 111 [313]. In the presence of Tween 20, the lead complex with meso-tetra(4-methoxyphenyl)porphyrin can be formed in 20 min at pH 12, the complex presents an absorption maxima at 467 nm and allows lead determination in the range of 0.1- 8 μg/10 mL [314]. The reaction between the malachite green and iodide and lead affords a ternary complex which when used with a previous preconcentration with a cationic resin, chelex 100, permits it to obtain a LOD of 25 μg/L [315]. A better LOD can be reached using a new chromogenic reagent, the 2- (2 - sulfo- 4 -acetyl(phenylazo)- 7 -(2, 4, 6 - trichlorophenylazo)- 1. 8 -dihydroxynapthalene-3,6-disulfonic acid; a 1:2 blue

complex with an absorption maxima of 654 nm has been obtained, and the LOD was 0.63 μg/L with a RSD of 2.6% [316]. Using the preconcentration with mercaptosephadex (MS-50) and the reaction of Pb(II) with dibromohydroxyl-phenylporphyrin a LOD of 1.4μg/L is possible [317]. The cetyl tri-Me-ammoniumbromide (TMAB) has been used in a different complex in order to increase the sensitivity; the complex was the Pb(II)-2,6,7.trihydroxy-9-(3,5-dibromo-4-hydroxyphenyl)fluorone [318] and Pb(II) salicylfluorone [319], obtaining in both cases a LOD of the order of a few μg/mL. The reagent benzil α-monoxime isonicotinoyl hydrazone has been used for the determination of lead in water, the complex presents an absorption maxima at 405 nm with a molar absorptivity of $1.18.10^4$ [320]. A similar molar absorptivity is given by the complex of lead with 4-(2′-thiazolylazo)-6-formyl-resorcinol, Beer's law is therefore obeyed in the range 0-8 μg/25 mL [321]. Surfaces of sulfhydril cotton fiber have been used to preconcentrate lead which is subsequently eluted into water where it is determined by silver nitrate-gelatin-ethanol solution at 420 nm following hydride generation with KBH_4; a very low LOD of $0.4.10^{-4}$μg /L was obtained [322]. Derivative spectroscopy has been applied for lead determination using liquid-liquid extraction of a ternary ion association complex Pb with 1-10-phenanthroline and Rose Bengal into chloroform; the procedure is simple, rapid and allows the determination of lead as low as 20 μg/L in sea water [323].

FAAS again has been used for lead determination in different types of water but the sensitivity is not sufficient for direct determination and a preconcentration step is necessary. Liquid-liquid extraction has been proposed using different reagents; Table 6 shows the different extraction systems proposed and the LOD obtained. Numerous preconcentration systems based on solid extraction or sorption have been proposed, and Table 7 include some examples with their analytical characteristics.

Table 6
Extraction system to preconcentrate the lead from water samples

Complexant reagent	Extraction solvent	LODμg/L	Reference
APDC	MIBK	3.1	[324]
TTA	Dichlorobenzene	-----	[325]
Dithizone	Chloroform	X 5	[326]
DDTC	MIBK	2.9	[327]
DDTC	Chloroform, CCl_4	2.3	[328]
APDC	MIBK	0.9	[329]
Benzylamine ,pelargonic acid	Decane	< 5.0	[330]
DDC	Chloroform	5 ng	[331]
Kbuxanthate	----	----	[332]
APDC	MIBK (Knotted reactor)	8	[333]

Table 7
Preconcentration system for lead in water

Preconcentration system	Analytical Characteristics	Reference
2-aminothiazole modified silicagel	Recovery ≈ 100%	[334]
Chromotrope2R-coated Amberlite XAD-1180	RSD < 10%	[335]
Phenylpiperazine dithiocarbamate	Recovery :90-100% Enrichment factor:400	[336]
Amberlite XAD-2 loaded with PAN	Preconcentrate factor :50	[337]
Crown ether	LOD : 1μg/L	[338]
o-o-diethylphosphorodithioic acid ammonium salt	Recovery > 95% RSD < 8%	[339]
Octadecyl-bonded SiO_2membrane disk with hexadentate Schiff base (1,8-bis(salicylaldiaminato)-3,6-dioxaoctene)	LOD : 0.7 μg/L	[340]
Polymer-fixed cryptand	LOD : 11.5 μg/L	[341]
Dithizone immobilized on sodium dodecylsulfate coated alumina	Preconcentration factor:200 RSD : 1.3%	[342]
DDTC retained on a $C_{18}SiO_2$	Preconcentration factor :100	[343]
Diaion HP-20 (1-nitroso-2-napthol complex)	LOD : 2.0 μg/L Recovery > 95%	[344]
Amberlite XAD-2 and Amberlite XAD-7	LOD : 2.44 –7.87 ng/mL	[345]
DDTP and sorption on a C_{18} bonded SiO_2	LOD : 7μg/L	[346]
Liq.membrane system of dicyclohe-Xyl-18-crown-6 Span-80, propa netriol,CCl_4 and $Na_4P_2O_7$	Enrichment Fcator:45 Recovery > 99% RSD: 1.1%	[347]
Cellulose with phosphonic acid and carboxymethyl groups	LOD : 0.17 μg/L	[348]
4-(4`-nitrophenylazo)-1-napthol and cetyltrimethylammonium bromide on silica gel	LOD:2 μg/L	[349]
Amberlite XAD-2 with salicylacid	Preconcentration factor:140	[350]
Sulfhydryl cotton	LOD: 0.86 μg/L RSD:0.6%,Recov:99-108%	[351]
Silochrome SG-80 modified with 2-aminothiazole	LOD: 0.03 mg/L	[352]
Cellulose ion exchanger cellex P	LOD: 0.15 μg/L	[353]

Preconcentration can be also be carried out using coprecipitation; thus the precipitation of lead with NH_3 onto the inner walls of a knotted reactor and the elution with 1M nitric acid allows a detection limit of 7.5 μg/L with a RSD of 2.9% [354]. La phosphate has been used as a coprecipitant for preconcentration of Fe(II) and Pb(II), giving a detection range between 5 and 400 μg/L of Pb [355]. $CaCO_3$ has also been used as a coprecipitant reagent leading to recoveries between 93.4 and 96.9%, RSD of 2.9-4.1% and with a linear range of 0.25-3.0 mg/L [356].

In order to increase the sensitivity of the flame, the use of the slotted quartz tube has been proposed for lead determination in water, obtaining a simple and rapid method with a linearity of 0-100 μg/L [357], and with a LOD of 1.9 μg/L [358]; this sensitivity can be improved by using derivative atom trapping flame atomic absorption spectrometry (DAT-FAAS), obtaining a LOD of 0.27 μg/L [359]. The modification of a commercially available slotted-tube atom trap (STAT) and a single silica tube water-cooled atom trap (WCAT) produces an improvement in sensitivity, obtaining a characteristic concentration of 1.042 ng/mL. Moreover, the slotted quartz tube has also been used with preconcentration methods, thus with the use of a FIA-FAAS with slotted quartz-tube with a column with poly(8-HQ-HSO_3) chelate resin it is possible to obtain a LOD of 1μg/L [360]; and with the use of a sulhydryl cotton fiber it is possible to obtain a sensitivity 2-3 times greater than with conventional FAAS [361].

Hydride generation-atomic absorption spectroscopy (HG-AAS) has also been used to determine lead in water samples [362,363], with the use of the flow injection (FI) mode it is possible to obtain 12 times higher sensitivity than in batch mode [364], which is the best generation media in terms of both sensitivity and freedom from interferences from the mixture lactic acid-$K_2Cr_2O_7$. The use of FI also allows one to work at a lower $NaBH_4$ concentration and a sampling frequency of 180 samples/hour. The use of derivative hydride-generation atomic absorption spectrometry (DHG-AAS) improves 26 times the sensitivity of conventional HG-AAS, giving a LOD of 9.6 μg/L [365]. HG has also been used with atomic fluorescence spectroscopy to obtain better detection limits [366].

ETAAS can be used for the direct determination of lead in water using an adequate chemical modifier. A comprehensive comparison of the analytical characteristic of the most frequently used modifiers has been carried out studying $NH_4H_2PO_4$, Pd, Pd-Mg, Pd-$NH_4H_2PO_4$, Mg-$NH_4H_2PO_4$, Ni-$NH_4H_2PO_4$, and Ni-Sr- $NH_4H_2PO_4$. The Pd- based modifiers behave very similarly except that sensitivity using Pd- $NH_4H_2PO_4$ is slightly lower. The use of Ni-Sr-$NH_4H_2PO_4$ raises the maximum tolerable pyrolysis temperature to 1300°C, which is significantly higher than with the other modifier. The permissible amounts of Cl^- and SO_4^{2-} interference are higher with the Ni-Sr-

$NH_4H_2PO_4$ modifier. The characteristic mass (m_o) was 6.1, 8.8, 7.9, 11.6, 10.5, 7.0 and 4.5 pg, respectively, whereas it is 12.1 pg without modifiers. The Ni-Sr-$NH_4H_2PO_4$ modifier performs better that the others [367]. The modifier Ni-$NH_4H_2PO_4$ has been used by various authors, obtaining different sensitivities: m_o of 3.4 pg and a LOD of 0.038 μg/L for a 20 μL sample [368], a m_o of 7 pg and a LOD of 0.14 μg/L for a 10 μL sample or (with NaOH) a m_o of 3.4 pg and a LOD of 0.038 μg/L for a 20 μL sample [369]. With the mixture $Co(NO_3)_2$-$NH_4H_2PO_4$ it is possible to obtain a LOD of 0.03 μg/L [370], and with the mixture ammonium vanadate plus sodium molybdate the LOD was 0.7 μg/L [371]. The mixture Ni(II)-ammonium tartrate using multiple sample injection gives a LOD of 0.1 μg/L [372]. H_3PO_4 has also been proposed as a chemical modifier for Pb leading to a LOD of 2.0 μg/L [373] and the mixture H_3PO_4-$NH_4H_2PO_4$ gives a LOD of 0.3 μg/L [374]. Lanthanum, $LaCl_3$ and HNO_3 or $La(NO_3)_3$ have been proposed as a modifier added to the aqueous samples or to coat the graphite tubes [375-376]; the most important advantage of this modifier is the suppression of interferences of Cl^- and SO_4^{2-}. It must be noted that the more important interferences in lead determination by ETAAS are the interferences caused by $MgCl_2$ and Na_2SO_4. The use of the L'Vov platform decreases the sulfate interference, but not $MgCl_2$ interference [377]; the best modifier to control this interference is La-HNO_3 acid mixture.

In order to increase the sensitivity and eliminate interferences, different preconcentration systems have been used. The adsorption on activated C in an NH_4Cl–NH_3 medium gives a LOD of 7.5 ng/L [378]. With the use of macrocyclic ligand immobilized on a silica gel support a LOD of 20 ng/L can be obtained [379]. The collection on activated carbon impregnated with 1,2-cyclohexanedienedioxime (Ac-Dox) at pH 8.0 yields a LOD of 0.075 μg/5cm^3 [380]. The use of chelating resin beads is possible in two forms: the metal can be eluted from loaded beads with acid for ETAAS analysis or the beads can be slurried in acid solution for direct ETAAS measurement. The method distinguishes between unstable Pb complexes and free Pb ions and it is possible to detect sub ppb levels [381]. Different extraction systems have been proposed: extraction with ammonium tetramethylene-dithiocarbamate [382], extraction with DDTC-benzene [383] and extraction with 1-phenyl-3-methyl-4-decanoyl-5-pyrazolone [384]; however, these systems do not present special advantages. Other preconcentration systems used were Ga(III)-phosphate coprecipitation with a LOD of 0.095 ng/cm^3 [385] and flotation with hydrated iron(III) oxide and iron (III) tetramethylenedithiocarbamate which gives a LOD of 0.30 μg/dm^3 [386].

ICP-AES has been used for the simultaneous determination of lead and other trace elements in water, but, in general, the detection limits are not adequate and the use of a preconcentration system is necessary. Different

chelating resins were used. Silica gel functionalized with methylthiosalicylate (TS-gel) and elution with EDTA produces a LOD for lead of 15.3 ng/mL with a RSD of 0.9% and a sample frequency of 24/hour [387]. With the Amberlite XAD-7 resin a LOD in μg/L was obtained [388] and with the chelating resin carboxymethylated polyethyleniminelpoly(methylenepolyphenylen)isocyanate (CPPI) a LOD of 0.6 μg /L can be reached [389]. Another approach was the preconcentration by coprecipitation; in this way, the coprecipitation with dithiophosphates produces an enrichment factor of 4-40 [390], whereas the coprecipitation with $In(OH)_3$ at pH 9.5 produces an increase of 240-fold [391]. On the other hand, electrochemical preconcentration with Hg film electrodes and the subsequent electrothermal vaporization and inductively coupled plasma atomic emission spectroscopy gave detection limits of a few μg /L [392].

ICP-MS can be used for the direct determination of lead in water. The direct isotope ratio measurement of ultratrace lead in waters by double focusing ICP-MS with an ultrasonic nebulizer and a desolvation unit was used to measure $^{206}Pb/^{207}Pb$, $^{206}Pb/^{208}Pb$ and $^{207}Pb/^{208}Pb$ isotope ratio for Pb concentrations 1-1000 ng/l. The most important problems with these determinations were the serious instrument contamination and the memory effects for lead [393]. To avoid the problems of the matrix, the preconcentration of the water is recommended; in this way, iminodiacetate resin [394] and chelex 100 [395] has been used, obtaining good results for both resins. Another preconcentration system is electrothermal vaporization (ETV) which has been used for Co, Ni, Cu and Pb in water samples. The efficiency of this preconcentration system is 100-fold and it is therefore possible to determine these heavy metal ions at pg/mL to ng/mL levels [396].

Molecular fluorescence has been used for lead determination in water using different fluorescent reagents. Thus the quenching effect of lead of the fluorescence of meso-C4-methyl(oxyphenyl)porphyrin (TMOPP) at pH 9.5 produced a LOD of $8.5.10^{-8}$ M [397]. The iodide and rhodamine B system allows measurement of concentrations between 0.5 μg Pb/mL [398]. A lower concentration range, 0.003-0.5 μg/mL, can be analysed with the solvent extraction of the ion-pair formed between the eosinate anion and the cationic complex of Pb(II) with 18-crown-6; this method is exceptionally selective for lead [399]. Another fluorescent system is the N-vinylcarbazole-acrylic acid copolymer and cetyl tri-methylammonium bromide surfactant [400], and the ion pair of the lead complex with kryptofix222BB and Eosin Y; using the last system a LOD of 6 μg/L can be obtained [401].

Flame atomic fluorescence spectrometry has also been used for the direct determination of lead in water samples. The samples acidified with 1% nitric acid were introduced directly into an N-shielded air-H or air-acetylene flame. The LODs were 2.5 μg/L and 7 μg/L for each type of flame, respectively [402].

ASV has been used for simultaneous metal determination in waters including the lead determination. Different types of electrodes and conditions were used, obtaining different detection limits. With a glassy C electrode modified with novel calixarene, a LOD of $6.1.10^{-9}$ M can be obtained; metal ions such as Hg(II), Ag(I) and Cu(II) can interfere, but these interferences can be eliminated with KSCN [403]. With a new modified C paste electrode the LOD was $9.0.10^{-10}$M after 6 minutes of accumulation [404]. The use of the adsorptive accumulation of 2′,3,4′,5,7-pentahydroxyflavone (morin) complexes of Cu, Zn and Pb into a hanging Hg drop electrode allows a LOD of 0.08 ng Pb/mL [405], and the complexes with pyrogallol red into a hanging Hg drop electrode gave a LOD of 0.06 ng Pb/mL [406]. The dithizone-modified glassy C electrode was used to detect trace levels of Cd(II) and Pb(II), obtaining a LOD for lead of $7.0.10^{-10}$M [407]. With the use of a 1-(2-pyridylazo)-2-napthol (PAN) drop-coated modified screen-printed carbon electrode (PAN-SPCE), lead determination is possible after 5 minutes of preconcentration with a LOD of 15 ng/mL [408]. The determination of lead at the nanomolar level by Square-Wave Anodic-Stripping-Voltammetry (SWASV) without removal of oxygen has been extended to the sub-nanomolar concentration range. The detection limit has been lowered to 0.05 nM (10ng/L) by using a method of differences at 60 seconds electrodeposition. In this method the analytical signal is the difference between the voltammogram of the sample and that obtained with no electrolysis, recorded sequentially. Because the presence of surfactants distorts the SWASV, in order to ensure surfactant-free solutions, pretreatment of the samples has been performed, including digestion with nitric and sulfuric acids, evaporation to dryness and heating at 650°C [409]. The LOD is limited by the purity of the reagents used in the digestion procedure. The presence of humic acids (HA) and other surface-active substances in a sample decrease the reliability of lead determination by SWASV. The implementation of thin-layer chromatography prior to SWASV minimizes the effect of these interferents by physical separation of lead from the sample matrix prior to analysis [410]. Potential stripping voltammetry has been proposed for the simultaneous determination of Zn, Cd and Pb in a 0.017 M sulfuric-nitric mixed acid medium by isochronous mercury plating method. The stripping potential peak for lead was –0.46 v (vs SCE); and the LOD was 0.7 ng/mL [411]. Cu(II), Sb(III), Bi(III) and Pb(II) were cathodically deposited to a hanging Hg drop electrode (HMDE) from a 1.0 M HCl medium in a flow cell followed by quantification by differential pulse anodic stripping voltammetry in a malonic acid/HCl stripping medium, obtaining LOD at the level of 10^{-10} M [412]. A very sensitive Cathodic Stripping Voltammetry method for the determination of lead with adsorptive collection of complexes with alcein Blue(8-(N,N-bis(carboxymethyl)aminomethyl)-4-methylumbelliferone onto a hanging mercury drop electrode was developed, obtaining a LOD of 0.04 nM after 1 min collection [413]. The interference of

iron can be used for the analysis in situ of lead in water; for this a submersible probe with a flow-through cell and a Hg film or a Hg drop electrode was used. The simultaneous determination of Cu, Pb, Cd and Zn is possible in oxygenated waters [414]. On the other hand, the voltammetric techniques (differential pulse polarography, anodic stripping voltammetry, and differential pulse anodic stripping voltammetry) can distinguish between dissolved and particulate lead (II) concentrations. The presence of colloids of FeOOH,MnO_2 and SiO_2 have no effect on the electrochemistry reversibility of the Pb(II) redox system [415].

Potentiometry can be used in portable instruments for copper(II) and lead(II) determination. To perform this determination, water samples of 400 μL were added to 200 μL of matrix modifying solution consisting of HCl,$CaCl_2$, Hg(II), ethanol, Triton X-100 and Bi(III) as internal standard. The lead is determined in the range 1-50 μg/L in 3 minutes [415].

6. QUALITY CONTROL AND REFERENCE MATERIALS FOR LEAD ANALYSIS

Quality control can be defined as the specific set of activities intended to examine both the analytical process and its results in terms of quality. Controlling quality in this respect entails continuously checking laboratory organization, equipment, reagents and the sample custody chain, among others, which lead to statistical control and to achieving the accuracy demanded from the measurement process. Equipment calibration, blank analyses, the use of suitable reference materials and skilled personnel, and close surveillance of every operation by the person in charge of the work team are typical components of quality control schemes. Statistical control (checking for measurement reproducibility) is the primary prerequisite, even above accuracy. In fact, irreproducible measurements can hardly be accurate. The International Organisation for Standardisation (ISO) defines accuracy as "The closeness of the agreement between the test results and the accepted reference value" [416].

To check the accuracy of an analysis, three alternatives are possible: 1) comparison of the results with results obtained by a different method, 2) comparison with other laboratories, and 3) the use of reference materials with known concentrations of the analyte.

A Refererence Material (RM) is defined as: "A material or substance of one or more properties which are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials" [417].

A Certified Reference Material (CRM) is defined as "A reference material accompanied by a certificate one or more of whose property values are certified by a procedure which establishes traceability to an accurate realisation of the

unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence" [418].

The use of CRMs is the easiest way to achieve accuracy. Certified reference materials of good suppliers link the user's results to those of the international scientific community. Additionally, they enable the user to verify his performance at any desired moment.

For lead determination, different reference materials for different matrices and with different concentration levels are available. These reference materials are supplied by different organizations such as: Bureau Communitarie de Reference (BCR); the International Atomic Energy Agency (IAEA); the Laboratory of the Government Chemist (LGC); the National Institute of Standards and Technology (NIST), etc. It is possible to look for these reference materials in the "Virtual Institute of Reference Materials" (VIRM) [419]. The central mission of this Virtual Institute is to be a knowledge network and a facility to encourage the interaction between all stakeholders in the field of reference materials (certified reference materials, quality control materials) for analysis. VIRM is a non-profit organization registered in Luxembourg. Tables 8-12 give a list of the reference materials with the concentration levels and the corresponding provider (first column), for environmental, biological and clinical matrices.

Another possible way to control the accuracy of the results is by participation in Intercomparison Programs of Quality Control. These programs are normally organized by the same organizations that produce the reference materials, and it is possible to find a great variety of programs for the different matrices of environmental materials or for biological samples.

Table 8
Reference materials for lead in biological samples

RM-Code	Analyte	Matrix	Value
AMI B1701	Pb, lead	Trace metals in human blood	0.28 µmol/L, certified
AMI B1702	Pb, lead	Trace metals in human blood	0.97 µmol/L, certified
AMI B1703	Pb, lead	Trace metals in human blood	2.30 µmol/L, certified
BCR-194	Pb, lead	Bovine blood	126 µg/L, certified
BCR-195	Pb, lead	Bovine blood	416 µg/L, certified
BCR-196	Pb, lead	Bovine blood	772 µg/L, certified
ERM-CE194	Pb, lead	Bovine blood	126 µg/L, certified
ERM-CE195	Pb, lead	Bovine blood	416 µg/L, certified
ERM-CE196	Pb, lead	Bovine blood	772 µg/L, certified

Table 9
Reference materials for lead in plants and animals

RM-Code	Analyte	Matrix	Value
BCR-060	Pb, lead	Aquatic plant	63.8 mg/kg, certified
BCR-061	Pb, lead	Aquatic plant	64.4 mg/kg, certified
BCR-670	Pb, lead	Lemna minor ,aquatic plant	2.06 mg/kg, non-certified
BCR-278	Pb, lead	Mussel tissue	2.00 mg/kg, certified
ERM-CE278	Pb, lead	Mussel tissue	2.00 mg/kg, certified

Table 10
Reference materials for lead in sediments

RM-Code	Analyte	Matrix	Value
BCR-320	Pb, extractable	River sediment	30 mg/kg, non-certified
BCR-320	Pb, total	River sediment	42.3 mg/kg, certified
BCR-667	Pb	Estuarine sediment	31.9 mg/kg, non certified
BCR-701	Pb, extraction step 1	Lake sediment	3.18 mg/kg, certified
BCR-701	Pb, extraction step 2	Lake sediment	126 mg/kg, certified
BCR-701	Pb, extraction step 3	Lake sediment	9.3 mg/kg, certified
CRM008-050	Pb	Soil/sediment	95.3 mg/kg, certified
CRM015-050	Pb	Sediment	15 mg/kg, certified
CRM016-050	Pb	Sediment	14.1 mg/kg, certified
LGC6137	Pb, extractable	Estuarine sediment	73.0 mg/kg, certified
LCG6139	Pb	River clay sediment	176mg/kg, non certified
LCG6139	Pb , extractable	River clay sediment	160 mg/kg non certified
LGC6156	Pb	Harbour sediment	1685 mg/kg non certified
LGC6187	Pb	River sediment	77.2 mg/kg, certified
METRANAL 1	Pb, extractable	River sediment	82.4 mg/kg, non certified
METRANAL 1	Pb, total	River sediment	93.2 mg/kg, non certified
RM9	Pb	Sediment	25-300 mg/kg, non certified

Table 11
Reference materials for lead in soils

RM-Code	Analyte	Matrix	Value
BCR-141R	Pb, aqua regia soluble	Calcareous loam soil	51.3 mg/kg, certified
BCR-141R	Pb, total	Calcareous loam soil	57.2 mg/kg, certified
BCR-142R	Pb, aqua regia soluble	Light sandy soil	25.7 mg/kg,,certified
BCR-142R	Pb, total	Light sandy soil	40.2 mg/kg, certified
BCR-143R	Pb, aqua regia soluble	Sewage sludge amended soil	174 mg/kg, certified
BCR-143R	Pb, total	Sewage sludge amended soil	179.7 mg/kg, certified
BCR-483	Pb, extracable acetic acid	Sewage sludge amended soil	2.1 mg/kg, certified
BCR-483	Pb,extractable EDTA	Sewage sludge amended soil	229 mg/kg, certified
BCR-484	Pb, extractable, acetic acid	Sewage sludge amended soil	1.17 mg/kg, certified
BCR-484	Pb, extractable, EDTA	Sewage sludge amended soil	47.9 mg/kg, certified
BCR-690	Pb	Calcareous soil	181 mg/kg, non certified
BCR-700	Pb, extractable acetic acid	Organic-rich soil	4.85 mg/kg, certified
BCR-700	Pb, extractable EDTA	Organic-rich soil	103 mg/kg, certified
BRM#01	Pb(DIN ISO11466 extractable)	Soil	340 mg/kg, non certified
BRM#02	Pb(DIN ISO11466 extractable)	Soil	353 mg/kg, non certified
BRM#03	Pb(DIN ISO11466 extractable)	Soil	1000mg/kg, non certified
CRM005-50	Pb	Soil	89.2 mg/kg, certified
CRM008-050	Pb	Soil/sediment	95.3 mg/kg, certified
CRM020-050	Pb	Soil	5110 mg/kg, certified
CRM021-100	Pb	Soil	145000 mg/kg, non certified
CRM022-030	Pb	Soil	415 mg/kg, certified
CRM023-050	Pb	Soil	214 mg/kg, certified
CRM024-050	Pb	Soil	15.7 mg/kg, certified
CRM025-050	Pb	Soil	1450 mg/kg, certified
CRM026-050	Pb	Soil	25.6 mg/kg, certified
CRM027-050	Pb	Soil	51.9mg/kg, certified
CRM028-050	Pb	Soil	10.4 mg/kg, certified
CRM030-050	Pb	Soil	7.13 mg/kg, certified
CRM033-050	Pb	Soil	60.0 mg/kg, certified
CRM036-050	Pb	Soil	132 mg/kg, certified
CRM037-050	Pb	Soil	118 mg/kg, certified
CRM038-050	Pb	Soil	128 mg/kg, certified

CRM039-050	Pb	Soil	178 mg/kg, certified
CRM040-050	Pb	Soil	120 mg/kg, certified
CRM042-050	Pb	Soil	182 mg/kg, certified
CRM043-050	Pb	Soil	150 mg/kg, certified
CRM044-050	Pb	Soil	75.9 mg/kg, certified
CRM045-050	Pb	Soil	42.8 mg/kg, certified
CRM046-050	Pb	Soil	45.3 mg/kg, certified
CRM202-225	Pb	Superfund soil	48.5 mg/L, certified
CRM204-225	Pb	Superfund soil	4,51 mg/L, certified
CRM206-225	Pb	Superfund soil	2.16 mg/L, certified
CRM207-225	Pb	Superfund soil	2.76 mg/L, certified
CRM208-225	Pb	Soil	2.14 mg/L, certified
CRM209-225	Pb	Soil	61.4 mg/L, certified
CRM210-225	Pb	Soil	133 mg/L, certified
CRM211-225	Pb	Soil	1.48 mg/L, certified
CRM212-225	Pb	Soil	0.06 mg/L, non certified
CRM213-225	Pb	Soil	4.9 mg/L, certified
CRM214-225	Pb	Soil	0,32 mg/L, certified
CRM215-225	Pb	Soil	1.93 mg/L, certified
CRM216-225	Pb	Soil	0,624 mg/L, certified
CRM217-225	Pb	Soil	1.75 mg/L, certified
CRM218-225	Pb	Soil	3.92 mg/L, certified
CRM219-225	Pb	Soil	0,786 mg/L, certified
CRM221-225	Pb	Soil	1.55 mg/L, certified
CZ 7001	Pb, extractable	Light sandy soil	24.1 µg/g, certified
CZ 7001	Pb, total	Light sandy soil	43.8 µg/g, certified
CZ 7002	Pb, extractable	Light sandy soil	35.5 µg/g, certified
CZ 7002	Pb, total	Light sandy soil	58.9 µg/g, certified
CZ 7003	Pb, extractable	Clay loam soil	25.2 µg/g, certified
CZ 7003	Pb, total	Clay loam soil	33.5 µg/g, certified
CZ 7004	Pb, extractable	Soil	83.1 µg/g, certified
CZ 7004	Pb, total	Soil	93.4 µg/g, certified
ERM-CC690	Pb	Calcareous soil	181 mg/kg, non certif
LGC6135	Pb	Brick works soil	411 mg/kg, certified
LGC6135	Pb, leachable	Brick works soil	391 mg/kg, certified
LGC6138	Pb	Coal-carbonisation site soil	490 mg/kg, non certified
LGC6141	Pb	Soil contaminated with clinker/ash	75.8 mg/kg, non certified
LGC6144	Pb	Gas works-contaminated soil	196 mg/kg, non certified
LGCQC3003	Pb	Contaminated soil 1	76 mg/kg, certified
VKI-Loam Soil A	Pb	Loam soil	31.4 mg/kg, certified

Table 12
Reference materials for lead in waters

RM-Code	Analyte	Matrix	Value
BCR-403	Pb, lead	Seawater	0.117 nmol/kg, certified
BCR-609	Pb, lead	Ground water	1.63 μg/kg,certified
BCR-610	Pb, lead	Ground water	7.78 μg/kg, certified
CRM002-100	Pb, lead	Water waste	5 mg/kg, non certified
LGC6010	Pb, lead	Hard drinking water	95 μg/L, certified
LGC6016	Pb, lead	Estuarine water	196 μg/kg,certified
LGC6017	Pb, lead	Rainwater-roof run-off	1 μg/L, certified
LGC6019	Pb, lead	River water-river Thames	5.2 μg/L, certified
LGC6021	Pb, lead	Soft drinking water	23.7 μg/L,certified
RM3A	Pb, lead	Clear water	5-95 μg/L, non certified
RM3B	Pb, lead	Waste water	10-3000 μg/L, noncertified
RM51	Pb, lead	Waste water	1.1-13 μg/kg, non certified
RM7	Pb, lead	Saline water	4-9 μg/L, noncertified
VKI-LL1	Pb, lead	Waste water	10.02 μg/L, certified
VKI-LL2	Pb, lead	Recipient water	20.3 μg/L, certified

7. SPECIATION OF LEAD

7.1. General aspects

The term "speciation" is defined by the International Union for Pure and Applied Chemistry (IUPAC) as follows [420]:

Chemical Species. Chemical element: Specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.
Speciation analysis. Analytical Chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.
Speciation of an element: Speciation. Distribution of an element amongst defined chemical species in a system.
Fractionation. Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g. size, solubility) or chemical (e.g. bonding reactivity) properties.

It is not always possible to determine the concentrations of the different chemical species that add up to the total concentration of an element in a given matrix. Often, chemical species present in a sample are not stable enough to be determined as such. During the measurement procedure, the partitioning of the element among its species may be changed. Moreover, the large number of individual species makes it impossible to determine the exact speciation. The aim therefore is to identify various classes of the elemental species.

Lead species

The most important group of species is the organolead compounds (R_nPbX), which are quite toxic. The more substituted the organic chain the higher the toxicity. Organolead compounds are very labile and easy transformations take place between them. In the environment, the tetraalkyllead compounds (TAL) are degraded by sunlight and atmospheric constituents (such as ozone and the hydroxyl radical) into trialkyllead ions [421-423], whereas in biological systems dealkylation occurs through reactions with thiol groups in proteins and enzymes [424,425]. Further conversion finally yields inorganic lead via dialkyllead intermediates [421,426]. Methylated lead species are less toxic than the corresponding ethylated compounds, but are more stable, volatile and have longer half-lives [421-426].While the major source of TAL in the environment is due to vehicular exhaust fumes, there are indications that lead can be methylated by biologically mediated mechanisms [427-430], but due to the reportedly low efficiency of such processes, the natural background level of methyllead species is insignificant. Nevertheless, such observations illustrate the bioavailability of lead in the environment, and the need to be able to monitor

individual alkyllead species in order to study the chemical cycles of such compounds and assess exposure risks.

7.2. Sampling, storage and pretreatment

The various types of samples have different requirements regarding sampling, storage and pretreatment procedures, and hence will be treated separately.

7.2.1. Environmental samples

For total lead determination in water samples, it is normal to acidify the sample in order to prevent losses by adsorption on the walls of the sample vessel. However, acidification changes the physicochemical distribution of lead species and must be not used prior to speciation. The sample should be analyzed immediately, or must be stored in previously cleaned teflon or polyethylene containers. Using properly cleaned containers, unacidified natural water samples may be stored at 4°C, in the dark, for up to 3 months without any measurable changes in the distribution of lead species [431-434].

An unresolved question is whether or not the water sample must be filtered. If the sample is not filtered, changes in the distribution of lead can occur due to adsorption and desorption processes at particle surfaces; moreover the risk of sampling error is increased, due to inhomogeneity in the distribution of particles in the water column. The filtration may introduce errors; variable concentration values might be obtained if some lead is initially associated with colloids in the sample and lead species in solution can be retained by colloids and particles trapped on the filter. To avoid changes during filtration, the use of bubbling nitrogen through the sample solution in the filter holder unit has been recommended [435]. To avoid rupture of phytoplankton, which could lead to elevated trace metal concentrations, the pressure difference across the filter should not exceed 26 KPa and a slight nitrogen overpressure is advantageous for filtering, which minimizes overloading and maintains the redox conditions in the sample.

Humic and fulvic acids are the predominant organic constituents present in river water. Metals, including lead, interact and form humic complexes which may exist alone or be associated with colloidal or suspended particles. The presence of humic complexes reduces the metal uptake by organisms and is an important factor in speciation work. The humic complexes can be separated using columns of XAD-2 resin or DEAE-Sephadex A-25 anion exchanger. Lead is desorbed with nitric acid and is determined by a suitable technique, such as ETAAS or ASV [434,437]. The separation of lead complexes can be performed at the sample collection site and the columns or filtrates transferred to the laboratory. This avoids contamination problems and changes in speciation which could occur if samples had to be stored prior to separation.

The trialkyllead species are present in natural waters and can be determined by high performance liquid chromatography (HPLC). Samples must be stored at 4°C in the dark to avoid photochemical degradation. Due to the low levels, off-line preconcentration is required. The TAL species may be extracted from water samples using organic solvent [438]. It is recommended to perform the extraction in the sample collection bottle since TALs are adsorbed on vessel walls, and to add the organic solvent as soon as possible after collection, as TALs decompose rapidly in aqueous solutions [439]. TALs decompose to inorganic lead via trialkyllead.

The solid-phase extraction methods (SPE) have been applied to the sampling of TALs from water samples, the Amberlite IR-120 resin [440] and a resin containing diethyldithiocarbamate groups [441] have been used to preconcentrate these compounds.

In the sampling of solid materials, their heterogeneity and the complexity of interactions with their surroundings (water, air) are important. It is necessary to minimize alterations in lead speciation resulting from changes in the environmental conditions of the system. Sub-surface soils and sediment are isolated from the influence of air and water and these materials must be protected from the atmosphere during sampling. Although there is not much information about these materials, the freezing of anoxic sediments has been shown to cause very little change in the fractionation pattern and may be a useful storage procedure [442]. Immediate analysis is recommended for surface soils and sediments as storage may significantly change the distribution of lead species. However, some form of pretreatment and storage is involved, wet storage at room temperature, air dried or oven dried, which may affect the results of speciation. Soil and sediment samples contain interstitial waters which can be removed by centrifugation, but this procedure is not recommended for speciation studies, because the interstitial waters contain the most mobile lead phase in equilibrium with the solid. During centrifugation, active sites freed on the surface may selectively re-adsorb some of the water-soluble metals resulting in a change in the speciation [443].

Particulate matter suspended in natural waters or in atmospheric aerosol can be collected by filtration; the filters must be cleaned previously with acid.

To determine alkyllead species, extraction with organic solvents such as hexane or benzene is recommended. In recent years, however, Supercritical Fluid Extraction (SFE) has been developed because it has these advantages: a) the use of non-aggressive chemicals in extraction, minimising the risk of analyte decomposition; b) continuous removal of analyte from the extraction cell, reducing redistribution problems; and c) reduction in the use of organic solvents.

7.2.2. Biological samples

In comparison to environmental samples the speciation of lead in biological samples has received little attention, probably due to the limited amount of material available (blood, biopsy samples), the complexity of the sample matrices and the analytical difficulties to perform speciation.

In the case of serum samples it is very important to avoid hemolysis during blood collection and serum separation. This is necessary because 10% of the lead concentration in the whole blood is situated in the serum, while the remaining 90% is concentrated in the cells. In the serum, lead seems to elute in the fraction ascribed to ceruloplasmin and ferritin.

The blood samples used to study the tetralkyl and ionic alkyllead can be hemolysed by freezing (-20°C) for at least 24 hours, and samples in such conditions are stable for extended periods [444,445]. On the other hand, other authors [446] have shown that the concentration of trimethyl and dimethyllead in blood are stable at 4°C for one week, at –20°C for two months and at –70°C for one year.

To ensure the stability of the species during transport, a polystyrene box with dry ice was used, which preserved the species' integrity for 2 days. The stability of these organolead compounds has also been investigated in an aqueous solution [447]. Solutions containing 500 mg/L of trimethyllead and triethyllead were stable for 3 months, but UV irradiation produced rapid decomposition of these lead species. Dimethyl and diethyllead species decomposed less rapidly under similar conditions.

Urine is a difficult matrix due to its very variable composition and concentration. The urine sample should be freshly collected to avoid problems with precipitation and filtered using a 0.45 μm filter [448]. The levels of the alkyllead species are very low; a preconcentration step using extraction or derivatization procedures (the same used for alkyllead determination by gas chromatography) is necessary.

For speciation studies the tissue must be homogenized and afterwards digested using tetramethylammonium hydroxide (TMAH) [449] or enzymatic hydrolysis with lipase and protease [450].

7.3. Analytical techniques for lead speciation

To perform lead speciation it is necessary to use an analytical technique with a good separation capacity and a specific detector for lead, normally an atomic detector. On the other hand, groups of species can be identified on the basis of gross behavioral differences and physicochemical properties. Such speciation schemes are operationally defined, and their limitations should be clearly understood.

7.3.1. Electrochemical methods

Electrochemical methods have been employed in the study of the bioavailability and toxicity of various chemical species of trace metals. The technique most used is ASV, because the preliminary preconcentration step provides the lowest detection limits. In order to determine the different chemical forms of lead in a water sample, ASV must be combined with a speciation scheme. One example of a speciation scheme is shown in the Table 13 [451]. In this scheme, the labile fraction (fraction 2) is regarded as bioavailable and in some cases was highly correlated with metal toxicity. In the case of lead, the lipid-soluble fraction may also be significant in terms of toxicity, due to the presence of alkyllead species.

Table 13
Speciation scheme for lead in natural water samples using ASV

Subsample	Pretreatment	Physicochemical form
1	Add HNO_3 to 0.05M+0.1%H_2O_2.UV for 8 h adjust pH to 4.7 with acetate buffer	Total
2	For fresh waters, buffer with 0.025M acetate to pH 4.7.Seawater untreated	ASV-labile
3	UV in presence of 0.1%H_2O_2 at natural pH	Organically bound (by difference 3-2)
4	Pass through ion exchange column containing chelex 100 resin	Very strongly bound
5	Extract with hexane-20%butanol.Retain aqueous phase and treat as in 1	Lipid-soluble (by difference 1-5)

Other speciation schemes can be used to study the different species in soils and sediments. The procedures proposed by Tessier [452] and by the BCR [453] allow the study of lead associated in the different fractions of the soil and sediment. To determine lead in the different fractions, it is possible to use electrochemical methods or atomic spectroscopy methods.

7.3.2. Chromatography–spectroscopic methods

High performance liquid chromatography (HPLC) has a number of advantages for lead speciation studies, e.g., minimal preparation of the liquid samples and separation at ambient or slightly elevated temperatures avoiding thermal decomposition risks for unstable species; non-volatile and inorganic lead compounds can be separated by HPLC; a large variety of stationary phases are available and ion-exchange, normal and reverse phases, as well as gel permeation chromatography allow the separation of ions, organometallics of low and high molecular mass as well as metal-protein compounds.

It is possible to use spectrophotometry as a detector, but a post-column chemical reactor is required. The trialkyllead species were determined at 515 nm in HPLC effluent after decomposition into dialkyllead by iodine and formation of 4-(2-pyridylazo)resorcinol, PAR complexes [454]. For application to urine [455] and natural water [456] a preliminary, off-line, solid-phase enrichment was necessary together with an on-line pre-column concentration step. These procedures are necessary, since detection limits are in the ng range, and the sample must be cleaned to eliminate potentially interfering concomitants which also form PAR complexes.

More common is the use of atomic detectors, such as FAAS, ETAAS, HG-AAS and ICP-AES. Direct coupling of HPLC to detectors using either FAAS or ICP-AES is fairly straightforward due to the continuous flow of both systems. However the sensitivities of these detectors, particularly the flame are not sufficient for lead speciation in most sample types of interest. The difficulty in coupling HPLC to FAAS is the balance of optimal flow rates between HPLC and FAAS. The common flow rate for HPLC is 1-1.5 mL/min, but that of FAAS is much higher. An additional solvent can be introduced into the nebulizer at the end of the HPLC column, but this produces sample dilution. Another possibility is to attach a Teflon funnel to the nebulizer or to introduce a small T-piece into the transfer line. Another disadvantage of this coupling is the efficiency of sample introduction; only around 5% of the sample could be introduced into the flame. Another problem is that the dispersion is not only in the HPLC column but also in the interface tube and FAAS detector, which decreases the sensitivity and resolution. The system HPLC-FAAS has been used in the lead speciation using a μBondapak C18 [457], and a Chelex 100 [458] as the chromatographic column, obtaining LODs of 10 ng and 0.17 ppb, respectively.

In order to increase the sensitivity of the flame, the use of the hydride generation has been proposed. After HPLC separation, the eluent is introduced into the hydride generator and mixed first with HCl, then with 1-5%$NaBH_4$ solution. The gaseous hydrides formed in the reaction coil are separated in a gas-liquid separator, introduced by inert gas flow into the heated quartz absorption cell and detected by AAS.

The use of ETAAS is desirable to increase the sensitivity, but this technique presents the problem of the discrete sampling mode of ETAAS. Normally, a fraction collector is used to assemble discrete samples of column effluent (50-500 μL) prior to subsequent ETAAS determination [459]. This system has been used to study the inorganic lead species in urine [460]. Another possibility is that the HPLC eluent can be continuously passed through a small volume with aliquots being withdrawn and injected into the graphite tube periodically [461]. The major limitation of this approach is that only well-separated species can be distinguished. The use of a thermospray interface allows the continuous introduction of the mobile phase of the HPLC and this

system can be used for lead speciation, although a limitation is the relatively low (0.2 mL(min maximum)) effluent flow rate. Organolead compounds in the concentration range 0.25-8 mg/L were selectively determined in the presence of inorganic lead by FI-HG-ETAAS [462], a carrier stream containing HCl and EDTA suppressed the inorganic lead signal for concentrations up to 10 mg/L.

Quartz T-tube atomic absorption spectrometry (QTAAS) has been used as an ionic alkyllead specific detector for HPLC using a thermospray-microatomizer interface [463]. This system has been used to study alkyllead species in water, soil and sediments. The effluent was introduced via a fused silica capillary transfer line through a heated (700-1000°C) side arm on the stem of the quartz tube atomizer and burned in the presence of oxygen. In another paper the ionic alkyllead species were converted into their volatile tetralkylderivatives using post-column ethylation [464]; these derivatives were then transferred to an electrically heated QT atomizer. The use of a post column reactor to obtain volatile lead derivatives and a quartz T-tube is a better method than coupling the HPLC to AAS detectors. The expansion problem during heating to atomization temperatures is avoided. The use of the quartz T-tube is preferable to a graphite tube due to the longer optical path length, because this produces a higher sensitivity.

The introduction of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) has presented new possibilities for element specific HPLC detection. The system HPLC-ICP-MS offers an unmatched performance for the detection and determination of non-volatile metallospecies. An important advantage of this coupling is the compatibility of the chromatographic effluent flow rate and the liquid flow rate required for stable pneumatic nebulization; interfacing of the two techniques is straightforward as the interface is very simple: a piece of narrow bore tubing connects the outlet of the LC column with the liquid flow inlet of a pneumatic nebulizer. Post-column addition of an internal reference element allows correction for changes in plasma conditions and other fluctuations in sensitivity. Nevertheless, some problems can occur due to the introduction of the mobile phases into the ICP [465,466]. The mobile phases generally use organic solvents, salts in buffer solutions and ion-pairing reagents. ICP-MS tolerates lower concentrations of organic solvents than ICP-AES. The buffer concentration used in exchange chromatography (usually > 0.1 M) causes signal suppression or enhancement and can cause blockage of the nebulizer and the sampling cone as well as erosion of the sampling cone and the skimmer [466]. High amounts of organic solvents decrease plasma stability (even leading to plasma extinction) and deposition of carbon on the torch. Water-cooled spray chambers and introduction systems equipped with a desolvation unit are therefore used to reduce the amount of solvent introduced into the plasma. A limitation of the coupling HPLC-ICP-MS is the low analyte transport efficiency to the plasma (>5%) which is inherent to pneumatic nebulization. This can be improved using other nebulizers such as the ultrasonic nebulizer on the direct

injection nebulizer (DIN) [467]; this is a microconcentric pneumatic nebulizer positioned inside the central tube of the ICP torch. The DIN interface presents a low dead volume, short wash-out times and no memory effects; peak broadening can be minimized and transport efficiency can approach 100% with mobile phase flow rates up to 0.1 mL/min. HPLC-ICP-MS has been used to study inorganic, trimethyl and triethyllead with absolute LOD in the range of 25-87 pg [468]. Using the DIN nebulizer for the same three lead forms, a LOD of about 0.2 pg has been obtained in urine [469]. Size exclusion HPLC-ICP-MS has been applied to study the speciation of lead in wine samples, observing that the lead is bonded to a major biomolecule of about 10 Kda [470] present in all wine samples and one to three minor compounds which do not depend on the wine sample.

Another coupling used in lead speciation studies is HPLC and laser enhanced ionization (LEI) spectrometry [471]. A pair of dye lasers induced two-step photoexcitation of the lead atoms produced in an air-acetylene flame prior to collisional ionization; applying a potential across electrodes in the flame the current resulting from the lead ions is measured. This coupling has been used to study the lead species in Reference Material NIST SRM 1566a Oyster Tissue; the triethyllead was the species detected, the absolute LOD obtained was 20 pg. This LOD is poorer than for HPLC-DIN-ICP-MS. For this reason and due to the high cost of this instrumentation, this HPLC-LEI coupling has been infrequently used.

The coupling of gas chromatography (GC) with atomic detectors is a powerful instrumentation used in speciation analysis and this hyphenated technique has been extensively used in recent years. The principal advantage over HPLC is that the gas stream emerging from the column can be introduced to flame AAS avoiding the problems associated with the overall insensitivity of the flame. If the gas emerging from the GC column can be introduced directly into the flame by passing the nebulizer, an increase in sensitivity is obtained. The main disadvantage of this GC-FAAS technique is that many of the organometallic compounds of interest are non-volatile and therefore must be derivatized prior to GC determination. The samples must also be thermally stable and not break down at the oven temperature used in GC. The transfer lines between both instruments must be heated in order to prevent analyte condensation.

Packed and capillary columns have been used for the separation of organic lead species by GC, the capillary columns being more popular because they provide much better resolution. The atomic absorption detectors used were the QT-AAS [471], and ETAAS [472], and a variety of simple interfaces have been described. Detection limits obtained by GC-AAS for organolead species vary widely depending of the type of atomizer used and the nature of the column. To obtain better detection limits, it is recommended to use: a) capillary

columns, because these provide higher resolution; and b) an atomizer of the quartz tube type which generally results in higher sensitivity than graphite tube systems due to the greater cell lengths.

Emission detectors have the principal advantage of their multielemental character, and the most used were the coupling GC-MIP-AES [473-481] and GC-ICP-MS [482]. GC is advantageous for coupling to MIP-AES. Firsly, the analytes can be introduced quantitatively into the plasma in gaseous form and no nebulization and drying is necessary. Secondly, GC separation can use He as the carrier gas, and this gas is ideal for helium MIPs. The interface is a simple heated transfer line with low dead volume. Some applications of the GC-MIP-AES system were the study of organolead compounds in snow samples using propylation with sodium tetrapropylborate derivatization [483], the determination of organolead compounds in tap water and peat with in situ butylation with tetrabutykammonium tetra-butylborate for derivatization [484], the study of organolead compounds in gasoline using multicapillary GC [485] and the study of the alkyllead contamination in wines [481]. Coupling GC-ICP-MS has been applied [482] for Hg, Pb and Sn speciation. For lead speciation in atmospheric particulate matter, butylation was selected for derivatization after the extraction of the different organolead compounds into hexane using DDTC. Detection limits in the low fg range were obtained for trimethyl, dimethyl, triethyl and diethyllead. The measurement of lead isotope ratios on the chromatographic peaks could be used to asses lead sources.

Mass spectrometry, MS, has been used as a detector for GC. The system GC-MS is commercially available and permits determination of the exact identity of the detected species, being useful in confirming the structure of derivatized lead compounds [472]. The use of isotope dilution MS also facilitates the monitoring and corrections for potential decomposition of lead species during either the sample collection step or the subsequent analytical procedure [486]. GC-MS has been applied for the detection of atmospheric TALs collected on a solid sorbent consisting of a mixture of Tenax and Porapak [487], to the determination of trimethyllead in urban dust [488], and for the ionic alkyllead compounds in rainwater [489].

In recent years, supercritical fluid chromatography (SFC) has grown in popularity as an alternative separation technique to HPLC and GC. SFC is a hybrid technique of HPLC and GC, combining some of the best features of each, i.e., the high diffusion coefficient of GC with the solubility properties of HPLC. Thermally labile, non-volatile and high mass molecular compounds (compounds difficult to separate by GC) can easily be separated by SFC. SFC is faster than HPLC due to the lower viscosity of the mobile phase and high diffusion coefficients of the analyte. The most common mobile phase is CO_2 to which a variety of modifiers can be added to optimize the solvating strength for the species. The analyte species are chromatographed with the mobile phase in the

supercritical state (7 MPa, 40°C for CO_2). The most common coupling is SFC-ICP-MS. The mobile phase changes from the supercritical fluid to the gaseous state before entering the plasma. The decompression is accomplished in an interface by implementing a restrictor connected to the end of the analytical column. Nebulizer and spray chamber can be eliminated. The application of SFC-ICP-MS to speciation studies has recently been revised [490]. The number of applications has been limited due to the popularity and the wide availability of detailed information on HPLC and GC separations. Moreover, the most common mobile phase, CO_2, is not ideal for most organometallic compounds. Elution problems and high interaction with the stationary phase are problems related in terms of polarities of the mobile phase and analyte compounds. Organic modifiers or formation of nonpolar metal complexes from polar organometallic compounds has been used to solve these problems. SFC-ICP-MS in single-ion monitoring mode has been used for lead speciation, obtaining a LOD in the range of 0.5 to 10 pg (as lead) [491], and further application to environmental samples is to be expected in the near future.

REFERENCES

[1] A. Bermejo, in El Plomo, presencia y efectos. Métodos de análisis. Universidad de Santiago de Compostela, Santiago de Compostela, Spain, 1982.

[2] K. Tsuchiya, in Lead in Toxicology of metals, vol. 2, National Technical Information Service, Springfield, VA, 977, 243.

[3] G. Moris, M. Patriarca, M. P. Carrieri, G. Fondi and F. Taggi, Ann. Ist. Super. Sanitá, 25 (1989) 423.

[4] S. Caroli, A. Alimonti, E. Coni, F. Petruci, O. Senofonte and N. Violante, Critical Reviews in Anal. Chem.,24 (1994) 363.

[5] W. Lockeretz, Science, 180 (1973) 1080.

[6] J. Rosmanith, H. J. Einbrodt and T. Gordon, Zentralbl. Bakteriol. Parasitenkd. Infektionskr.Hgy.,161 (1975) 125.

[7] E. Coni, A. Alimonti, G. B. Bolis, E. Cristallini and S. Caroli, Trace Element. Med., 11 (1996) 86.

[8] M.M. Erickson, A. Poklis, G.E. Gantner, A.W. Dickinson and L.S. Hillman, Pediatric Research, 17 (1983) 779.

[9] K. Sumino, K. Hayakawa, T. Shibata and S. Kitamura, Arch. Environ. Health, 30 (1975) 487

[10] F. Nordberg, P.O. Wester and D. Brune, in Proceeding of International Symposium on the Control of Air Pollution in the Working Environment, Lyber Tryck, Stockholm (1978) 261.

[11] E.M. Ophus and E.A. Mylius, Bull. Environ. Contam. Toxicol., 18 (1977) 734.

[12] E.A. Mylius and E.M. Ophus, Bull. Environ. Contam. Toxicol., 17 (1977) 302.

[13] D.A. Skoog, F.J. Holler and T.A. Nieman, in Principles of Instrumental Analysis, Saunders College Publishing (eds.), Philadelphia, 1992.

[14] S. Stary in The Solvent Extraction of Metal Chelates, Pergamon Press, 1964.

[15] S.S. Cooper and J.O. Hibbits, J. Am. Chem. Soc., 75 (1953) 5084.

[16] J.J. Snyder, Anal. Chem., 19 (1947) 684.

[17] P.A. Clifford, J. Assoc. Offic. Agr. Chemist, 26 (1973) 26.
[18] W.M. McCord and J.W. Zemp, Anal. Chem., 27 (1955) 1171.
[19] S.L. Thompsett, Analyst, 81 (1956) 330.
[20] E. Berman, Am. J. Clin. Pathol., 36 (1961) 549.
[21] A.I. Vogel, in A Textbook of Quantitative Inorganic Analysis, Longman, London, 1978
[22] V. Armeanu, Revtachim., 19 (1968) 290.
[23] B.K. Puri and S. Burnam, Z. Anal. Chem., 286 (1977) 253.
[24] K.C. Srivastava, Chim. Analyt., 51 (1969) 28.
[25] G. Roebisch, Anal. Chim. Acta ,47 (1969) 539.
[26] C. Cabrera, Quim. Anal., 28 (1974) 38.
[27] E. Sebastiani, K. Ohls and G. Riemer, Fresenius Z. Anal. Chem., 264 (1973) 105.
[28] H. Berndt and E. Jackwerth, Spectrochim. Acta Part B, 30(1975) 169.
[29] H. Berndt and E. Jackwerth, At. Absorpt. Newsletter, 1515 (1976) 109.
[30] R.C. Fry, S.J. Northway and M.B. Denton, Anal. Chem.,50 (1978) 1719.
[31] A.S. Inglis and P.W. Nicholls, Mikrochim. Acta II, (1975) 553.
[32] D.C. Manning, At. Absorpt. Newsletter ,14 (1975) 99.
[33] D.L. Tsalev and I. Petrov, Anal. Chim. Acta, 111 (1979) 155.
[34] H.L. Kahn, At. Absorpt. Newsletter,7 (1968) 35.
[35] H.T. Delves, Analyst, 95 (1970) 431.
[36] J.F. Fernandez and H.L. Kahn, At Absorpt. Newsletter., 10 (1971) 1.
[37] R.D. Ediger and R.L. Coleman, At. Absorpt. Newletter, 11 (1972) 33.
[38] H.L. Kahn and J.S. Sebestyen, At. Absorpt. Newsletter, 9 (1970) 33.
[39] R.M. Dagnall and T.S. West,Talanta,11 (1964)1553.
[40] W.J. Price, Spectrochemical Analysis by Atomic Absorption, Heydon , London,1979
[41] H.L. Kahn and J.S. Sebestyen, Atom. Spectros., 9 (1970) 33.
[42] B. L'Vov, L.A. Pelieva and A.I. Sharnopolsky, Zh. Prikl. Specktrosk., 25 (1977) 395.
[43] B. L'Vov, Spectrochim. Acta, 33B (1978) 153.
[44] W. Frech and S. Johnsson, Spectrochim. Acta, 37B (1982) 1021.
[45] B. Welz, G. Schlemmer and J.R. Mudakavi, J. of Anal. Atom. Spectrom., (1988) 695.
[46] B. Welz, G. Schelemmer and J.R. Mudakavi, J. of Anal. Atom. Spectrom., (1988) 93.
[47] P. Bermejo Barrera, J. Moreda Piñeiro, A. Moreda Piñeiro and A. Bermejo Barrera, Atom. Spectrosc., 19 (1998) 100.
[48] W. Slavin, D.C. Manning and G.R. Carnrick, At. Spectrosc., 215 (1981) 137.
[49] B. L'Vov and G.N. Ryabchuk, Spectrochim. Acta, 37B (1982) 673.
[50] R.E. Sturgeon, D.F. Mitchell and S.S. Berman, Anal. Chem., 55 (1983) 1059.
[51] M.L. Kaiser, S.R. Koirtyohann and E.J. Hinderberger, Spectrochim. Acta, 36B (1981) 773.
[52] S.R. Koirtyohann, M.L. Kaiser and E.J. Hinderberger, J. Am. Off. Assoc. Chem., 65 (1982) 999.
[53] D.C. Manning and W. Slavin., Appl. Spectrosc., 37 (1983) 1.
[54] T.W. May and W.G. Brumbaugh, Anal. Chem., 54 (1982) 1032.
[55] T.C. Rains, T.A. Rush and T.A. Butter, J. Am. Off. Assoc. Chem., 65 (1982) 994.
[56] L.E. Wittmers, A. Alich and A.C. Aufarheide, Am. J. Clin. Path, 75 (1981) 80.
[57] M. Bengtsson, L. Danielsson and B. Magnusson, Anal. Lett., 12 (1979) 1367.
[58] K.C. Thompson, K. Wagstaff and K.C. Wheatstone, Analyst, 102 (1977) 310.
[59] H.V. Lagesson and L. Andrasko, Clin. Chem., 25 (1979) 1948.
[60] M.C. Halliday, C. Houghton and J.M. Ottaway, Anal. Chim. Acta, 119 (1980) 67.
[61] C.W. Fuller, At. Absorpt. Newsletter, 16 (1977) 106.
[62] T.M. Vickrey, G.H. Harrison and G.J. Ramdow, At. Spectrosc., 1 (1980) 116.
[63] D.J. Hodges and D. Skelding, Analyst, 106 (1981) 299.

[64] P.W.J.M. Boumans in Inductively Coupled Plasma Emission Spectrometry. Part I: Methodology, Instrumentation and Performance, Part II: Applications and Fundamentals, John Wiley, New York, 1987.
[65] M. Thompsom and J.N. Walsh, Handbook of Inductively Coupled Plasma Spectrometry, Blackie, Glasgow, 1989.
[66] A. Montaser and D.W. Golightly in Inductively Couples Plasmas in Analytical Atomic Spectrometry, VCH Publisher, Ins, New York, 1992.
[67] D.A. Skoog, F.J. Holler and T.A. Nieman, in Principles of Instrumental Analysis, Saunders College Publishing, 5th ed, Florida, 1998.
[68] K.E. Jarris, A.L. Gray and R.J. Houk, in Handbook of Inductively Coupled Plasma Mass Spectrometry, Blackie, New York, 1992.
[69] G.N. Bowers, J.D. Fassett and E. White, Anal. Chem., 65 (1993) 475R.
[70] J.R. Moody and M.J. Epstein, Spectrochim. Acta, 46B (1991) 1571.
[71] J.D. Fassett and P.J. Paulson, Ana. Chem., 61 (1989) 643A.
[72] C.C. Patterson and D.M. Settle in La Fleur PD ed, Accuracy in Trace Analysis: sampling, sample handling and analysis, vol 1. NBS Special Publication 422, Gaithersburg, MD, 1976.
[73] D.C. Paschal, K.L. Caldwell and B.G. Ting, J. Anal. Atom. Spectrom., 10 (1995) 367.
[74] R.J. Bowins and R.H. Menutt, J. Anal. Atom. Spectrom., 9 (1994) 1233.
[75] ESA Laboratories, Inc.Model 3010 B lead analyzer operating manual. Chelmsford, MA:ESA, Inc, 1994.
[76] S.M. Roda, R.D. Greenland, R.L. Bornschein and P.B. Hammond, Clin. Chem., 34 (1988) 563.
[77] L. Duic, S. Szechter and S. Srinivasau, Electroanal. Chem. Interfac. Electrochem., 41 (1973) 89.
[78] T.P. DeAngelis, R.E. Bond, E.E. Brooks and W.R. Heinemann, Anal. Chem., 49 (1977) 1792.
[79] K. Ichiro, F. Koichi and H. Shunichi, J. Applied Toxicol., 1 (1981) 295.
[80] K. Ichiro, F. Koichi and H. Shunichi, Sangyo Igaku, 23 (1981) 82.
[81] J.A. Cocho, J.R. Alonso Fernández and J. Fraga, Acta Científica Compostelana, 21 (1984) 285.
[82] J.R. Alonso Fernández, C. Parrado, J.A. Cocho, M.C. Castiñeiras and J.M. Fraga, Anales de Química, 87 (1991) 258.
[83] O. Nygren, C.A. Nilsson and A. Gustarsson, Analyst, 113 (1988) 591.
[84] J.F. Rosen and E.A. Trionidad, J. Lab. Clin. Med., 80 (1972) 567.
[85] N.P. Kubasik, M.T. Volosin and M.H. Murray, Clin. Chem., 18 (1972) 410.
[86] S.S. Quehee, T.J. McDonald and R.L. Bornschein, Microchem. J., 32 (1985) 55.
[87] K.G. Brodse and M.W. Routh, Clin. Biochem., 17 (1984) 19.
[88] D.K. Eaton and J.A. Holcombe, Anal. Chem., 85 (1983) 946.
[89] V.P. Garnys and L.E. Smythe, Talanta, 22 (1975) 881.
[90] H. Koizumi and K. Yasuda, Anal. Chem., 48 (1976) 1178.
[91] W.N. Anderson, P.M.G. Broughton, J.W. Dawson and G.W. Fisher, Clin. Chim. Acta, 50 (1974) 129.
[92] P.A. Pleban and K.H. Pearson, Anal. Lett., 12B (1979) 935.
[93] J.A. Ealy, N.E. Bolton, R.J. McElheny and R.W. Morrow, Am. Ind. Hyg. Assoc. J., 35 (1974) 566.
[94] P.D. Castilho and R.F.M. Herber, Anal. Chim. Acta, 94 (1977) 269.
[95] A.R. DelRosario, G.N. Guirguis, G.P. Perez, V.C. Matias, T.H.Li and C.P. FlesselInt., J. Environ. Anal. Chem., 12 (1982) 223.
[96] S.K. Giri, C.K. Shields, D.Littlejohn and J.M. Ottaway, Analyst, 108 (1984) 244.

[97] G. Edmonstone and J.C. VanLoon, Spectroscopy Letters, 17 (1984) 591.
[98] K.S. Subramanian, Prog.Anal.Spectrosc., 9 (1986) 237.
[99] K.S. Subramanian, At. Spectrosc., 9 (1988) 169.
[100] V.P. Garnys and L.E. Smythe, Talanta, 22 (1975) 881.
[101] K.G. Brodie and B.J. Stevens, J. Anal. Toxicol., 1 (1977) 282.
[102] V.P. Garnys and J.P. Matousek, Anal. Chem., 21 (1975) 891.
[103] M. Stoeppler, K. Brandt and T.C. Rains, Analyst, 103 (1978) 79.
[104] M. Stoeppler, C. Mohl, P. Ostapezuk, M. Goedde, M. Roth and E. Weidmann, Fresenius'Z. Anal. Chem., 317 (1984) 486.
[105] K.S. Subramanian, At. Spectrosc., 8 (1987) 7.
[106] D.J. Halls, Analyst, 109 (1984) 1081.
[107] T.L. Shuttler and H.T. Delves, Analyst, 111 (1986) 651.
[108] K.S. Subramanian and J.C. Méranger, Clin. Chem., 27 (1981) 1866.
[109] S.T. Wong, G. Strunc and F. Peter in S.S. Brown and J.Savory (eds) Chemical Toxicology and Clinical Chemistry of Metals, Academic Press, London, (1983) 57.
[110] M.Z. Asan and A. Kumar, Ind. J. Environ. Health, 25 (1983) 191.
[111] D.J. Hodges and D. Skelding, Analyst, 108 (1983) 813.
[112] V. Kurfürst, Fresenius'Z. Anal. Chem., 313 (1982) 97.
[113] E.J. Hinderberger, M.L. Kaiser and S.R. Koirtyohann, At. Spectrosc., 2 (1981) 1.
[114] S.R. Koirtyohann, M.L. Kaiser and E.J. Hinderberger, J. Assoc. Off. Anal. Chem., 65 (1982) 999.
[115] F.J. Fernandez and D. Hilligoss, Atom. Spectrosc., 3 (1982) 130.
[116] E. Pruszkowska, G.R. Carnrick and W. Slavin, Atom. Spectrosc., 4 (1983) 59.
[117] S. Constantini, R. Giordano and M. Rubbiani, Microchem. Journal, 35 (1987) 70.
[118] D.T. Miller, D.C. Paschal, E.W. Gunther, P.E. Stround and J.D. Angelo, Analyst, 112 (1987) 1701.
[119] C.G. Bruhn, J.M. Piworka, M.A. Jerardine, G.M. Navarrete and P.C. Maturana, Anal. Chim. Acta, 198 (1987) 113.
[120] M. Patriarca, M. Castelli, F. Corsetti and A. Manditto, Clin. Chem., 50 (2004) 1396.
[121] P. Parsons and W. Slavin, Spectrochim Acta, Part B: Atom. Spectrosc., 488 (1993) 925.
[122] M.Z. Hasan and A. Kumar, Indian J. of Environ. Health, 25 (1983) 191.
[123] F.J. Fernández and D. Hilligoss, Atom. Spectrosc., (1982) 130.
[124] N. Koga, Y. Hirai and K. Tomokuni, Nippon Eiseigaku Zasshi, 40 (1985) 636.
[125] Y. Seki, S. Kido, S. Imamiya and Y. Kisdi, Igaku to Seibutsugaku, 119 (1989) 191.
[126] K.E. Murphy and P.J. Paulsen, Fresenius'J. of Anal. Chem., 352 (1995) 203.
[127] P.Y. Wang and C.L. Li, Fenxi Huaxue, 8 (1980) 534.
[128] M. Jin, L.Wang and X. Chen, Huaxue Fence, 38 (2002) 293.
[129] G. Wu, N. Jin, A. Xu and L. You, Fenxi Huaxue, 14 (1986) 557.
[130] M. Nedeljkovic, L. Stojadinovic, V. Matovic and Z. Koriranac, Clin. Chim. Acta, 168 (1987) 169.
[131] C. Lui and Y. Zhang, Huanjing Huaxue, 1 (1982) 392.
[132] Epidemic Prevention Station of Xuzhou District, Huanjing Kexue, 3 (1982) 63.
[133] A. Bermejo Barrera, P. Bermejo Barrera and F. Bermejo Martínez, Laboratorio (Granada, Spain), 409 (1980) 13.
[134] H. Wang and Z. Wang, Huaxue Fence, 35 (1999) 323.
[135] L. Wuensch, Fresenius'Z. Anal. Chem., 310 (1982) 430.
[136] J. Yao and Y. Du, Huaxue Fence, 28 (1992) 102.
[137] N.K. Djane, I.A. Bergdahl, K. Ndung'u, A. Schutz, G. Johansson and L. Mathiasson, Analyst, 122 (1997) 1073.

[138] H. Berndt, S. Guecer and J. Messerschmidt, J Clin. Chem and Clin. Biochem., 20 (1982) 85.
[139] A. Huang, Z. Pan, W. Chang and B. Xu, Gongye Weisheng Yu Zhiyehing, 15 (1989) 38.
[140] R. Knutti in Atomspecktrom. Spureanal. Vortr. Kollog, Verlag Chem. Weinheim, Germany, (1982) 57.
[141] X. Q. Shan and Z.M. Ni, Canadian J. Spectrosc., 27 (1982) 75.
[142] T. Chen and D. Littlejohn, Analyst, 118 (1993) 541.
[143] S.K. Aggarwal, M. Kinter and D.A. Herold, Clin. Chem., 40 (1994) 1494.
[144] J. Song, Gongye Weisheng Yu Zhiyebing, 12 (1986) 298.
[145] J.L. Stauber and T.M. Florence, Anal. Chim. Acta, 237 (1990) 177.
[146] X. Sun, M. Zhang and S. Guo, Huaxi Yike Daxue Xuebao, 20 (1989) 324.
[147] M. Watts, B.J. Poulsen, L.L. Szabo, M.A. Kenny and W.Y. Lee, J. Anal. Toxicol., 5 (1981) 231.
[148] W. Mu and Y. Zhang, Gongye Weisheng Yu Zhiyebing, 14 (1988) 357.
[149] L. Sun and B. Wang, Fenxi Huaxue, 9 (1981) 679.
[150] D.L. Tsalev, L. Lampugnani, R. Georgieva, K.K. Chakarova and I.I. Petrov, Talanta, 58 (2002) 331.
[151] L.A. Milton, S.E. Collings and M.S. Johnson, Anal de Quím. International Edition, 94 (1998) 349.
[152] R. Chakraborty, S.S. Bhattacharyya and A.K. Das, Bull. Chem. Soc. of Japan, 66 (1993) 2233.
[153] J.E. Podoski, Anal. Chem., 52 (1980) 1147.
[154] B.T. Yaru, D. Bainok and G.M. Day, Atom. Spectrosc., 20 (1999) 33.
[155] S.C. Willie, Atom. Spectrosc., 15 (1994) 205.
[156] E. Luecker, J. Anal. Atom. Spectrom., 14 (1999) 1731.
[157] E. Luecker and S. Thorius-Ehrler, Fresenius'J. Anal. Chem., 346 (1993) 1072.
[158] E. Luecker, A. Rosopulo and W. Kreazer, Fresenius'J. Anal. Chem., 328 (1987) 370.
[159] A. Rosopulo and W. Kreuzer, Forrschritte in der Atomspektrometrischen Spureanalytik, 2 (1986) 455.
[160] J.F. Alder and M.C.C. Batoreu, Anal. Chim. Acta, 155 (1983) 199.
[161] E. Luecker, H. Koening, W. Gabriel and A. Rosopulo, Fresenius'J. Anal. Chem., 342 (1992) 941.
[162] G. Schlemmer, U. Voellkopf and B. Welz, in Heavy Met. Environ., Int.Conf., 5th, 2 (1985) 487.
[163] J.L.M. Boer and F.J.M.J. Maessen, Spectrochim. Acta, Part B: Atomic Spectrosc., 38B (1983) 739.
[164] E. Engstrom, A. Stenberg, S. Senioukh, R. Edelbro, D.C. Baxter and I. Rodushkin, Anal. Chim. Acta, 521 (2004) 123.
[165] S.F. Boulyga, J.S. Becker, A.F. Malenchenko and H.J. Dietze, Mikrochim. Acta, 134 (2000) 215.
[166] B. Benes, K. Jakubes, J. Smid and V. Sperackova, Biol. Trace Element Research, 75 (2000) 195.
[167] K.M. Lee, J. Appleton, M. Cooke, K. Sawicka-Kapusta and M. Damek, Fresenius'J. Anal. Chem., 364 (1999) 245.
[168] S. Sriramachari, A.K. Jain, S.K. Das, S. Rao and J.K. Sindhu, in Harmonization of Health Related Environmental Measurements using Nuclear and Isotopic Techniques, Proceeding of an International Symposium, Hyderobal, India, 1996.
[169] Y. Gelinas, J. Lafond and J.P. Schmit, Biological Trace Element Research, 59 (1998) 63.
[170] A. Ejima, C. Watanabe, H. Koyama and H. Satoh, J. Expe. Medicine, 178 (1996) 1.
[171] A. Cox, F. Keenan, M. Cooke and J. Appleton, Fresenius'J. Anal. Chem., 354 (1996) 254.

[172] S. Chen, Z. Zhang, H. Yu, W. Liu and M. Sun, Anal. Chim. Acta, 463 (2002) 177.
[173] J. Li, F. Lu, T. Umemura and K.I. Tsunoda, Anal. Chim. Acta, 419 (2000) 65.
[174] M.J. Campbell and H.T. Delves, J. Anal. Atom. Spectrom., 4 (1989) 235.
[175] B. Liu, S. Yuan and L. Ding, Fenxi Huaxue, 28 (2000) 126.
[176] E. Stryjewska, S. Rubel and A. Skowron, Chemia Analityczna (Warsaw), 39 (1994) 609.
[177] R.N. Khandekar and U.C. Mishra, Fresenius'J. Anal. Chem., 319 (1984) 577.
[178] J. Li, X. Li, Q. Ye, X. Tang, Y. Zhang and G. Yang, Fenxi Shiyanshi, 13 (1994) 62.
[179] P. Mader, J. Musil, E. Curdova, J. Koreckova and J. Cibulka, ChemickeListy, 81 (1987) 1190.
[180] A. Irquierdo, M.D. Luque de Castro and M. Valcarcel, J.Automatic Chem., 15 (1993) 121.
[181] A. Irquierdo, M.D. Luque de Castro and M. Valcarcel, Electroanalysis, 6 (1994) 894.
[182] C. Labar and L. Lamberts, Electrochim. Acta, 39 (1994) 317.
[183] A. Ide-Ektessabi, T. Kawakami, R. Ishihara, Y. Mizuno and T. Takeuchi, J. Electron Spectrosc and Related Phenomena, 137-140 (2004) 801.
[184] M.L. Carvalho and A.F. Marques, X-Ray Spectrometry, 30 (2001) 397.
[185] L. Benninghoff, D. von Czarnowski, E. Denkhaus and K. Lemke, Sprectrochim. Acta, Part B: Atomic Spectrosc., 52B (1977) 1039.
[186] K.P. Koutzenogii, T.I. Savchenko, O.V. Chankina, G.A. Kovals'kaya, A.I. Smirnove and L.P. Osiopora, Poverkhnost, 12 (2003) 14.
[187] M.L. Carvalho, J. Brito and M.A. Barreiros, X-Ray Spectrometry, 27 (1998) 198.
[188] J.D. Wallace and B.J. Thomas, Australasian Physical & Engineering Sciences in Medicine/Supported by the Australian College of Physical Scientists in Medicine and the Australasian Association of Physical Sciences in Medicine, 16 (1993) 118.
[189] F.M.G. Tack, S.P. Sing and M.G. Verloo, Agrochimica, 41 (1997) 182.
[190] E. Wieteska, A. Ziolek and A. Drzewinska, Chemie Analityczena (Warsaw), 42 (1997) 837.
[191] M.J. Knight and Argonne, I.L., USA, Arail. NTIS Report, 1980 (ANL/LRP-TM-18).
[192] J. Nieuwenhuize and C.H. Poley-Vos, Atomic Spectroscopy, 10 (1989) 148.
[193] J.L. Peral Fernández and J. Reyes Andrés, Anales de Bromatología, 39 (1987) 111.
[194] O. Duque Díaz, M. Catasus Portuondo, M. Granda Valdés, R. Rodríguez Chang and E. Handal, Quím. Anal., 15 (1996) 123.
[195] S.R. Sahito, T.G. Kazi, G.H. Kazi, M.A. Memon, Q. Shaikh, M.A. Jakhrani and G.Q. Shar, J. Chem.Soc.of Pakistan, 25 (2003) 201.
[196] G. Du Laing, F.M. Tack and M.G. Verloo, Anal. Chim. Acta, 497 (2003) 191.
[197] O. Sabina Rossini, H. Raitio and M.D. Mingorance, Commun. in Soil Science and Plant Anal., 34 (2003) 2913.
[198] A. Carlosena, D. Prada and E. Fernández, Quím. Anal., 13 (1994) 214.
[199] J. Azcue and A. Mudrolch, Inter. J. Environ. Anal. Chem., 57 (1994) 151.
[200] I. Novozamsky, V.J.G. Houba, J.J. VanderLee, R. van ECK and M.D. Mignorance, Commun. in Soil Science and Plant Anal., 24 (1993) 2595.
[201] B. Zunk, Anal. Chim. Acta, 236 (1990) 337.
[202] J. Borkowska-Burnecka, J. Wisz and W. Zyrnicki, Chemia Analityczna (Warsaw), 48 (2003) 115.
[203] J. Ruíz Jimenez, J.L. Luque García and M.D. Luque de Castro, Anal. Chim. Acta, 480 (2003) 231.
[204] C. Peña Farfal, A. Moreda Piñeiro, A. Bermejo Barrera, P. Bermejo Barrera, H. Pinochet Cancino and Ida de Gregori Hernández, Anal. Chim. Acta, 548 (2005) 183.
[205] N.S. Rajurkar and M.M. Damame, J. Radioanal. and Nuclear Chem., 219 (1997) 77.
[206] S. Tokalioglu, S. Kastal and A.A. Gnes, Inter. J. Environ.Anal. Chem., 80 (2001) 201.
[207] J. Fleurence and C. LeCoeur, Botánica Marina, 37 (1994) 555.

[208] S. Walas, E. Borowska, M. Herda, M. Herman and H. Mrowiec, Int. J. Environ. Anal. Chem., 72 (1998) 217.
[209] T. Balaji, P. Chiranjeevi and G.R.K. Naidu, Anal. Letters, 31 (1998) 1081.
[210] I. Kojima and S. Nomura, Anal. Sciences, 11 (1995) 17.
[211] K.W. Jackson, L. Ebdon, D.C. Webb and A.G. Cox, Anal. Chim. Acta, 128 (1981) 67.
[212] J.J. Van der Lee, E. Temminghoff, V.J.G. Houba and I. Novozamsky, Appl. Spectrosc., 41 (1987) 388.
[213] J. Medved, V. Stresko, J. Kubova and E. Chemielewska, Chemical Papers, 57 (2003) 169.
[214] M. Tuzen, Microchem. Journal., 74 (2003) 289.
[215] P.L. Teissedre, M.T. Cabanis and J.C. Cabanis, Analusis, 21 (1993) 249.
[216] D. Halls, C.Mohl and M. Stoeppler, Analyst, 112 (1987) 185.
[217] E. Vassileva, H. Baeten and M. Hoening, Fresenius'J. Anal. Chem., 369 (2001) 491.
[218] D. Baralkiewicz, M. Kozka, H. Gramowska, Tomaszewska and K. Wasinkiewicz, Inter. J. Environ. Anal. Chem., 84 (2004) 901.
[219] P. Vinas, N. Campillo, I. López García and M. Hernández Cordoba, Food Chem., 50 (1994) 317.
[220] R. Dobrowolski and J. Mierzwa, Fresenius'J. Anal. Chem., 346 (1993) 1058.
[221] F. Dolinsek, J. Stupar and V. Vrscaj, J. Anal. Atom. Spectrom., 6 (1991) 653.
[222] Z. Yu, C. Vandecasteele, B. Desmet and R. Dams, Mikrochim. Acta, 1 (1990) 41.
[223] I. Karadjova and M. Karadjo, Fresenius'J. Anal. Chem., 360 (1998) 246.
[224] L. Amoedo, J.L. Capelo, I. Lavilla and C. Bendicho, J. Anal. Atom. Spectrom., 14 (1999) 1221.
[225] D.C. Baxter and W. Frech, Fresenius'J Anal. Chem., 337 (1990) 253.
[226] V. Kos, B. Budic, V. Hudnik, F. Lobnik and M. Zupen, Fresenius'J. Anal. Chem., 354 (1996) 648.
[227] R.C. Munter, R.A. Grande, Ed: Barnes and R.M. Dev. At. Plasma Spectrochem. Anal., Proc. Int. Winter Conf., 1981, 653.
[228] A.P. Murphy, M. Coudert and J. Barker, J. Environ. Monitoring, 2 (2000) 621.
[229] A. Hokura, H. Matsuura, F. Katsuki and H. Haraguchi, Anal. Sciences, 16 (2000) 1161.
[230] K.A. Anderson, Atom. Spectrosc., 17 (1996) 30.
[231] J. Borkowska-Burnecka, Fresenius'J. Anal. Chem., 368 (2000) 633
[232] M.C. Valdés-Hevia y Temprano, M.R. Fernández de la Campa and A. Sanz Medel, Anal. Chim. Acta, 309 (1995) 369.
[233] S. Sparkes and L. Ebdon, Anal. Proceedings, 23 (1986) 410.
[234] Y. Okamoto, Fresenius'J Anal. Chem., 367 (2000) 295.
[235] P. Masson, D. Orignac, A. Vives and T. Prumet, Analusis, 27 (1999) 813.
[236] P. Masson, Spectrochim. Acta, Part B: Atomic Spectroscopy, 54B (1999) 603.
[237] S. Wu, X. Feng, A. Wittmeier and J. Xu, Trace Elements in Man and Animals, Proceedings of the International Symposium on Trace Elements in Man and Animals, 1997, 257.
[238] X. Feng, S. Wu, A. Wharmby and A. Wittmeir, J. Anal. Atom. Spectrom., 14 (1999) 939.
[239] I. Rodushkin, T. Ruth and A. Huhtasaari, Anal. Chim. Acta, 378 (1999) 191.
[240] S. Wu, X. Feng and A. Wittmeiu, J. Anal. Atom. Spectrom., 12 (1997) 797.
[241] M. Bettinelli, S. Spezia, U. Baroni and G. Bizzarri, Special Publication- Royal Society of Chemistry, 1997, 202 (Plasma Source Mass Spectrometry), 211.
[242] Y. Li, S. Jiang and S. Chen, Anal. Chim. Acta, 372 (1998) 365.
[243] S.F. Durrant, Analyst, 117 (1992) 1585.
[244] J. Li, F.Lu, T. Umemura and K.I. Tsunoda, Anal. Chim. Acta, 419 (2000) 65.
[245] J. Vacek, J. Petrek, R. Kizek, L.Harel, B. Klejdus, L. Trnkova and F. Jelen, Bioelectrochemistry, 63 (2004) 347.

[246] C. Locatelli, F. Fagioli, C. Bighi, S. Landi and T. Garai, Annali di Chimica, 74 (1984) 521.
[247] M.M.P.M. Neto and A. De Varennes, Plant and Soil, 154 (1993) 1.
[248] W. Jin, V.D. Nguyen, P.Valenta and H.W. Nurnberg, Anal. Letters, 30 (1997) 1235.
[249] M. Rozali bin othman, J.O. Hill and R.J. Magee, Fresenius'J. Anal. Chem., 326 (1987) 350.
[250] D.K. Ray, P.K. Nayak, T.R. Rautray, V. Vijayan and S. Jena, Indian Journal of Physics, B, 78B (2004) 103.
[251] Z. Tekin and M. Tan, Spectroscopy Letters, 32 (1999) 661.
[252] J. Iranova, R. Djingova and I. Kuleff, Journal of Radioanal. and Nuclear Chem., 238 (1998) 29.
[253] N. Marmiroli, E. Maestre, G. Antonioli, C. Conte, P. Monciardini, M. Marmiroli and C. Muechino, Internat. J. Phytoremediation, 1 (1999) 169.
[254] A. Viksna and E.S. Lindgren, Proceeding of the European Conference on Energy Dispersive X-Ray Spectrometry (EDXRS-98), (1999) 257.
[255] J.R. Preer and G.B. Murchison, Environmental Pollution Series B: Chemical and Physical, 12 (1986) 1.
[256] M. Jamaluddin Ahmed and M.A. Mamun, Talanta, 55 (2001) 43.
[257] P. Krishna, R. Gopi, V.M. Biju, T. Rao, N. Prasada and G.R.K. Naidu, Chemia Analityczna (Warsaw, Poland), 49 (2004) 383.
[258] L. Elci, Z. Arslan and J.F. Tyson, Spectrochim. Acta, Part B: Atom. Spectrom., 55B (2000) 1109.
[259] J. Liu, H. Chen, X. Mao and X. Jin, International J. Environment. Anal. Chem., 76 (2000) 267.
[260] H. Chen, J. Lin and Y. Wang, Anal. Chim. Acta, 353 (1997) 181.
[261] R. Ma, W.V. Mol and F. Adams, Anal. Chim. Acta, 285 (1994) 33.
[262] R. Ma and F.Adams, Spectrochim. Acta, PartB: Atomic Spectros., 51B (1996) 1917.
[263] A.M. Aziz-Alrahman, M.A. Al-Hajjaji and I.Z. Al-Zamil, Interna. J. Environ. Anal. Chem., 15 (1983) 9.
[264] P. Sooksamiti, H. Geckeis and K. Grudpan, Analyst, 121 (1996) 1413.
[265] I. Havezov, Proceedings-Seminar on Atomic Spectrochemistry, 14th Pondabanske, Slovakia, (1998) 30.
[266] C.M. Lau, A.M. Ure and T.S. West., Bull. Chemical Society of Japan, 61 (1988) 79.
[267] C.M. Lau, A.M. Ure and T.S. West, Anal. Chim. Acta, 146 (1983) 171.
[268] M.W. Hinds and K.W. Jackson, J. Anal. Atom. Spectrom., 5 (1990) 199.
[269] M.W. Hinds, M. Katyal and K.W. Jackson, J. Anal. Atom. Spectrom., 3 (1988) 83.
[270] M.W. Hinds and K.W. Kackson, J. Anal. Atom. Spectrom., 3 (1988) 997.
[271] E.C. Lima, J.L. Brasil and A.H.D.P. Santos, Anal. Chim. Acta, 484 (2003) 233.
[272] T. Myohanen, V. Mantylahti, K. Koivernen and R. Matilainen, Spectrochim. Acta, Part B: Atomic Spectros., 57B (2002) 1681.
[273] M.W. Hinds, K.E. Latimer and K.W. Jackson, J. Anal. Atom. Spectrom., 6 (1991) 473.
[274] M.W. Hinds, K.W. Jackson and A.P. Newman, Analyst, 110 (1985) 947.
[275] M.W.Hinds and K.W. Jackson, Atom. Spectros., 12 (1991) 109.
[276] M. Tighe, P. Lockwood, S. Wilson and L. Lisle, Communications in Soil Science and Plant Analysis, 35 (2004) 1369.
[277] V. Sandroni, C.M.M. Smith and A. Donovan, Talanta, 60 (2003) 715.
[278] V. Sandroni and C.M.M. Smith, Anal. Chim. Acta, 468 (2002) 335.
[279] M. Bettinelli, G.M. Beone, S. Spezia and C. Baffi, Anal. Chim. Acta, 424 (2002) 289.
[280] A. Vaeisaenen, R. Suontamo, J. Silvonen and J. Raintala, Anal and Bioanal. Chem., 373 (2002) 93.

[281] M.E. Rybak and E.D. Salin, Applied Spectros., 55 (2001) 816.
[282] G. Zaray and T. Kantor, Spectrochim. Acta, PartB: Atomic Spectrosc., 50B (1995) 489
[283] S. Sparkes and L. Ebdon, Anal. Proceedings, 23 (1986) 410.
[284] L. Moenke-Balnkenburg, T. Schumann and J. Noelte, J. Anal. Atom. Spectros., 9 (1994) 1059.
[285] H. Elfering, J.T. Andersson and K.G. Poll, Analyst, 123 (1998) 669.
[286] M. Bettinelli, C. Baffi, G.M. Beone and S. Spezia, Atom. Spectrosc., 21 (2000) 60.
[287] W. Diegor, H. Longerich, T. Abrajano and I .Horn, Anal. Chim. Acta, 431 (2001) 195.
[288] P. Chattopadhyay, A. Fisher, S. Andrew, D.N. Henon and S.Hill, Mikrochim. Acta, 144 (2004) 277.
[289] B. Chen, C.A. Shand and R. Beckett, J. Environ.Monitoring, 3 (2001) 7.
[290] Y. Lee, C. Chang and S. Jiang, Spectrochim. Acta, Part B: Atom. Spectros., 58B (2003) 523.
[291] S.A. Baker, M.Bi, R.Q. Aucelio, B.W. Smith and J.D. Winefordner, J. Anal. Atom. Spectrom., 14 (1999) 19.
[292] S. Cehn and S. Jiang, J. Anal. Atom. Spectrom., 13 (1998) 1113.
[293] K.E. Murphy, E.S. Beary, M.S. Rearick and R.D. Vocke, Fresenius'J. Anal. Chem., 368 (2000) 362.
[294] R. Dolan, J. VanLoon, D. Templeton and A. Paudyn, Fresenius'J. Anal. Chem., 336 (1990) 99.
[295] V. Ettler, M. Mihaljevic and M. Komarek, Anal. and Bioanal. Chem., 378 (2004) 311.
[296] J. Kowalska, B. Krasnodebska-Ostrega and J. Golimowski, Anal and Bioanal. Chem., 373 (2002) 116.
[297] T.E. Edmonds, P. Guogang and T.S. West, Anal. Chim. Acta, 120 (1980) 41.
[298] K.B. Olsen, J. Wang, R. Setiagji and J. Lu, Environ. Science and Technol., 28 (1994) 2074.
[299] P. Madsen, D. Pheiffer and J. Soerensen, Anal. Chim. Acta, 151 (1983) 479.
[300] R.O. Kadara and I.E. Ibtisam, Anal and Bioanal. Chem., 378 (2004) 770.
[301] R.K. Dubey and B.K. Puri, Talanta, 42 (1995) 65.
[302] M.A. Taher, B.K. Puri and R.K. Bausal, Microchem. Journal, 58 (1998) 21.
[303] L.V. Koplitz, J. Urbanik, S. Harris and O. Mills, Environ. Science and Technol., 28 (1994) 538.
[304] R. Sitko, B. Zawisza, J. Jurezyk, F. Bubl and U. Zielonke, Polis. Journal of Environ. Studies, 13 (2004) 91.
[305] T.D. Hettipathirana, Spectrochim. Acta, Part B: Atomic Spectros., 59B (2004) 223.
[306] S. Clark, W. Menrath, M. Chen, S. Roda and P. Succop, Proceeding of International Symposium and Exhibition on Environmental Contamination in Central and Eastern Europe, Warsaw, Poland, (1998) 1313.
[307] C. Vanhoof, V. Corthouts, K. Tirez and N. DeBrucker, Proceeding of the International Conference on Strategies and Techniques for the Investigation and Monitoring of Contaminated Sites, Karlsruhe, Germany (2001) 357.
[308] C. Vanhoof, V. Corthouts and K. Tirez, J. Environ. Monitoring, 6 (2004) 344.
[309] A. Argyraki, M.H. Ramsey and P.J. Potts, Analyst, 122 (1997) 743.
[310] M.S. DiNezio, M.E. Palimeque and B.J. Fernández Band, Talanta, 63 (2004) 405.
[311] O.P. Shroeva, V.P. Dedkova and S.B. Sawin, J. Anal. Chem., 56 (2001) 1080.
[312] V. Kuban and R. Bulawa, Collection of Czechoslovak Chemical Communications, 54 (1989) 2674.
[313] V.K. Jain, R.A. Pandya, S.G. Pillai, Y.K. Agrawal and P.S. Shrivastav, Microchim. Acta, 147 (2004) 253.
[314] Z. Lian-wen, L. Yan-Qin, H. Shi-Tian and S. Yi, Guangpu Shiyanshi, 21 (2004) 24.

[315] R.B.R. Mesquita, S.M. Fernandes and A.O.S.S. Ranged, Talanta, 62 (2004) 395.
[316] G. Yong, D. Bingjun, L. Yongwen, C. Xijun and M. Shuangming, Mikrochim. Acta, 144 (2004) 257.
[317] L. Zaijun, Y. Yuling, J. Tang and P. Jiaomai, Talanta, 60 (2003) 123.
[318] D. Bin, Y. Jinher, W. Qin and C. Guohua, Anal. Letter, 35 (2002) 895.
[319] H. Ming and W. Yinghui, Lihua Jianyau Huaxue Fence, 29 (1993) 349.
[320] M. Ramesh, K.B. Chandrasekher and K.H. Redly, Bioinorganic, Physical, Theoretical & Anal. Chem., 39A (2000) 1337.
[321] Z. Yurui, J. Wanquan, J. Gu and L. Wei, Indian J. Chem. Section A: Inorg. Bio-Inorg., Phys, Theo. & Anal. Chem, 35A (1996) 1009.
[322] D. Haiyau, Y. Bing and W. Bingwu, Fushun Shiyou Xueyuau Xuebao, 15 (1995) 32.
[323] D. Nair, R. Sreevalsan, T. Rao, C.I.P. Iyer and A.D. Damodaran, Anal. Letters, 26 (1993) 523.
[324] J. Ai, D. Li and Z. Tang, Fenxi Kexue Xuebao, 17 (2001) 414.
[325] H. Hossain and T. Houjo, Fresenius'J. Anal. Chem., 361 (1998) 451.
[326] G.D. Mao, C.C. Foreback and J.W. Chu, Toxicol. And Environ. Chem., 63 (1997) 163.
[327] J. Jin, H. Chen and X. Shen, Fexi Huaxue, 24 (1996) 957.
[328] F.A. Chmilenko, A.N. Baklanov and N.N. Bezkrornaya, Izvestiya Vysshikh Uchebnykh Zavedenii, Khimiya I Khimicheskayo Tekhnologiya, 34 (1991) 34.
[329] N. Ybañez, R. Montoro, R. Catala and M.L. Cervera, Revista de Agroquímica y Tecnología de Alimentos, 27 (1987) 270.
[330] V.N. Savitskii, V.I. Peleshenko and V.I.Osadchii, Zhurnal Analiticheskoi Khimii, 42 (1987) 677.
[331] T.W. Brueggemeyer and J.A. Caruso, Anal. Chem., 54 (1982) 872.
[332] U.V. Naidu and G.R.K. Naidum Indian J. Environ. Protection, 11 (1991) 282.
[333] Y. Li, Y. Jiang, X.R. Yan, W.J. Peng and Y.Y. Wu, Anal. Chem., 74 (2002) 1075.
[334] I. Luiz de Alcantara, P. Roldan, M.A.L. Mangionte, G.R. Castro, C.C.F. Padilha, A. Florentino and P. Padilha, J. Brazilian Chemical Society, 15 (2004) 366.
[335] M. Soylak, S. Saracoglu and L. Elci, Trace Elements and Electrolytes, 20 (2003) 160.
[336] H. Cesur, Turkish Journal of Chemistry, 27 (2003) 307.
[337] P. Bermejo-Barrera, N. Martínez-Alfonso, C. Díaz-López and A. Bermejo-Barrera, Microchim. Acta, 142 (2003) 101.
[338] Y. Bakirioglu, S. Rio Segade, E.R. Yourd and J.F. Tyson, Anal. Chim. Acta, 485 (2003) 9.
[339] A.U. Karatepe, M. Soylak and L. Elci, Anal. Letters, 36 (2003) 797.
[340] M.R. Ganjali, F. Basiripour and M. Jalavati-Niasari, Canadian J. Anal. Sci. Spectros., 47 (2002) 41.
[341] A. Dantz Dirk, H.J. Busshmann and E. Schollmeyer, Vom Wasser, 89 (1997) 115.
[342] S. Dadfarnia, A.M. Shabani and H.D. Shirie, Bulletin of the Korean Chemical Society, 23 (2002) 545.
[343] L.F. Capitan Vallvey, O. Duque and N. Navas, Química Analítica, 20 (2002) 197.
[344] M. Soylak, L. Elci, Y. Akkaya and M. Dogan, Anal. Letters, 35 (2002) 487.
[345] P.K. Tewari and A.K. Singh, Talanta, 56 (2002) 735.
[346] S.M. Sella, A.K. Avila and R.C. Campos, Anal. Letters, 32 (1999) 2091.
[347] Q. Su, S. Zhang, F. Li and M. Wang, Kuangwu Yanshi, 19 (1999) 97.
[348] A.M. Naghmush, K. Pyrzynska and M. Trojanowicz, Talanta, 42 (1995) 851.
[349] P.B.J. Rychlovsky and E. Bazakasova, Collection of Czechoslovok Chemical Communications, 60 (1995) 76.
[350] R. Saxena, A.K. Singh and D.P.S. Rathore, Analyst, 120 (1995) 403.
[351] X. Liu and Y. Zhu, Huaxue Fence, 34 (1998) 131.

[352] N.E. Kuzmenko, N.V. Golovina, G.V. Kozyreva, V.A. Popkov and G.D. Brykina, Vestnik Moskovskogo Universiteta, Seriya 2 :Khimiya 31 (1990) 523.
[353] K. Brajter and K. Slonawske, Water Research, 22 (1988) 1413.
[354] Q.Y. Ye, Y. Li, Y. Jiang and X.P. Yan, Atomic Spectros., 23 (2002) 154.
[355] S. Kagaya, Z.A. Malek, Y. Araki and K. Hasegawe, Anal. Sciences, 18 (2002) 923.
[356] C. Li and D.Wang, Zhongguo Gonggong Weisheng, 16 (2000) 432.
[357] J. Miao, Guangpuxue Yu Guangpu Fenxi, 21 (2001) 859.
[358] K. Chi and Y. Gao, Fenxi Shiyanshi, 13 (1994) 52.
[359] H.W. Sun, L.L. Yang, D.Q. Zhang, W.X. Wang and J.M. Sun, Fresenius'J Anal. Chem., 358 (1997) 646.
[360] X. Zhang, Fenxi Huaxue, 28 (2000) 1493.
[361] M. Zhang and L. Yuan, Guangpuxue Yu Guangpu Fenxi, 11 (1991) 62.
[362] Y. Li, Huaxue Fence, 28 (1992) 11.
[363] R. Smith, Atomic Spectros., 2 (1981) 155.
[364] Y. Madrid, D. Chakraborti and C. Camara, Mikrochim. Acta, 120 (1995) 63.
[365] H. Sun, J. Ha, J. Sun, L. Yang and D. Zhabg, Fresenius'J. Anal. Chem., 371 (2001) 1154.
[366] F. Yu and T. Yu, Guangpuxue Yu Guangpu Fenxi, 20 (2000) 898.
[367] Y. Liang and Y. Xu, J. Anal. Atom. Spectrom., 12 (1997) 855.
[368] Y. Xu and Y.Z. Liang, Inter. J. Environ. Anal. Chem., 68 (1997) 369.
[369] Y. Xu and Y. Liang, J. Anal. Atom. Spectrom., 12 (1997) 471.
[370] T. Shirasaki, H. Nakamura and K. Hiraki, Bunseki Kagaku, 43 (1994) 1149.
[371] J.L. Manzoori and A. Saleemi, J. Sciences. Islamic Republic of Iran, 7 (1996) 243.
[372] I. Sekerka and J.F. Lechmer, Anal. Chim. Acta, 254 (1991) 99.
[373] W.K. Oliver, S. Reeve, K. Hammond and F.B. Basketter, J. Institution of Water Engineers and Scientists, 37 (1983) 460.
[374] M.Z. Hasan and A. Kumar, Proceeding of the Indian National Science Academy, Part A: Physical Sciences, 49 (1983) 654.
[375] I.S. Fletcher, Anal. Chim. Acta, 154 (1983) 235.
[376] M. Feuertein and G. Schlemmer, Atomic Spectros., 20 (1999) 149.
[377] P.R. Sthapit, J.M. Ottaway, D.J. Halls and G.S. Fell, Anal. Chim. Acta, 165 (1984) 121.
[378] X. Su, M. Wang, Y. Zhang, J. Zhang, H. Zhang and Q. Sin, Talanta, 59 (2003) 989.
[379] E. Hosten and B.Welz, Anal. Chim. Acta, 392 (1999) 55.
[380] A. Uzawa, T. Narukawa and T.O. Kutoni, Anal. Sciences, 14 (1998) 395.
[381] V.K. Ososkov, C.C. Chou and B.B. Kebbekus, Inter. J. of Environ. Anal. Chem., 69 (1998) 1.
[382] R.P. Mitcham, Analyst, 105 (1980) 43.
[383] E. Nakamura, H. Namiki and N. Sakurabayashi, Kogyo Yosui, 404 (1992) 9.
[384] Y. Sasaki, S. Koizumi, T. Toshikuni and A. Tamada, Denki Kagaku Kyokai, Gijutsu, Kyoiku Kenkyu Ronbunshi, 4 (1995) 79.
[385] S. Kagaya and J. Ueda, Bull. Chem.Society of Japan, 70 (1997) 1379.
[386] T.Stafilov and K. Cundeva, Glasnik na Hemicarite I Technolozite na Makedonija, 15 (1996) 93.
[387] M. Zougagh, A. García de Torres, E. Vereda Alonso and J.M. Cano Pavon, Talanta, 62 (2004) 503.
[388] R.G. Wuilloud, J.A. Salonia, J.A. Gasquez, R.A. Olsina and L.D. Martínez, Anal. Sciences, 17 (2001) 457.
[389] C. Yang, Z. Zhuang, X.Wang and S. Qin, Atomic Spectros., 15 (1994) 135.
[390] D.A. Atanassova, V. Stefanova and E.D. Russeva, Bulgarian Chemical Communications, 32 (2000) 177.
[391] M. Hiraide, T. Ito, M. Baba, H. Kawaguchi and A. Mizuike, Anal.Chem., 52 (1980) 804.

[392] T. Malinski, J. Fish and H. Matusiewicz, Proceeding-Water Quality Technology Conference, Vol Date 1986, 1987, 14 (Adv. Water Anal. Treat.) 347.
[393] F. Poitrasson and S.H. Dundas, J. Anal. Atom. Spectrom., 14 (1999) 1573.
[394] D.B. Taylor, H.M. Kingston, D.J. Nogay, D. Koller and R. Hutton, J. Anal. Atom. Spectrom., 11 (1996) 187.
[395] A. Itoh, T. Ozaki, K. Chiba and H. Haraguchi, Anal. Sciences, 13 (Suppl., Asia analysis IV) (1997) 7.
[396] T. Tanaka, Y. Ando, T. Saitoh and M. Hiraide, J. Anal. Atom. Spectrom., 17 (2002) 1556.
[397] L.P. Long, W. Lui, C.X. Lei and T.S. Zhong, Guangpu Shiyanshi, 21 (2004) 595.
[398] Z. Xu, Q. Man and X. Yu, Huanjing Wurau Yu Fangzhi, 7 (1985) 39.
[399] A. Sanz Medel, D. Blanco Gomis, E. Fuente and S. Arribas Gimeno, Talanta, 31 (1984) 515.
[400] G. Aodeng, Y. Sai, X. Sheng and L. Geri, Neimenggu Daxue Xuebao, Ziran Kexueban, 25 (1994) 575.
[401] E. Kaneko, J. Isoe, T. Iwabuchi, S. Hoshi, K. Akatsuka and T. Yotsuyanagi, Analyst, 127 (2002) 219.
[402] P.R. Sthapit, J.M. Ottaway and G.S. Fell, Analyst, 109 (1984) 1061.
[403] J.Q. Lue, X.W. He, X.S. Zeng, H.L. Zhang and Z.Z. Zhang, Chinese J. Chem., 21 (2003) 687.
[404] W-Y. Qu, Huagong Keji, 11 (2003) 47.
[405] E. Shams, A. Babaei and M. Soltaninezhad, Anal. Chim. Acta, 501 (2004) 119 .
[406] A. Ensafi, T. Khayamian and M. Atabati, Talanta, 59 (2003) 727.
[407] W. Huang, C. Yang and S. Zhang, Fenxi Huaxue, 30 (2002) 1367.
[408] W.F. Pickering, Water Research, 1 (1984) 1119.
[409] E. Kirova-Eisner, M. Brand and D. Tzar, Anal. Chim. Acta, 385 (1999) 325.
[410] S.C. Petrovic and H.D. Dewald, Anal. Letters, 31 (1998) 2077.
[411] W. Zhang, Huaxue Fence, 37 (2001) 499.
[412] F. Wang, S. Li, S. Liu, Y. Zhang and Z. Liu, Anal. Letters, 27 (1994) 1779.
[413] K. Yokoi, A. Yamaguchi, M. Mizumachi and T. Koide, Anal. Chim. Acta, 316 (1995) 363.
[414] U. Goerlach and C.F. Boutron, Anal. Chim. Acta, 236 (1990) 391.
[415] M.L.S. Goncalves, L. Sigg and W. Stumm, Environ. Science and Technol., 19 (1985) 141.
[416] D. Jagner, E. Shalin, B. Axelson and R. Ratana-Ohpes, Anal. Chim. Acta, 278 (1993) 237.
[417] Statistic-Vocabulary and Symbols-Part 1: Probability and General Statistical Terms, Revised ISO 3534, ISO-International Organisation for Standardisation, Geneva, 1993.
[418] Terms and Definitions used in connection with Reference Materials, ISO Guide 30, revised 1992, International Organisation for Standardisation, Geneva, 1992.
[419] Virtual Institute of Reference Materials, http://www.virm.net.
[420] D.M. Templeton, F. Ariese, R. Cornelis, L.G. Danielsson, H. Muntau, H.P. VanLeeuwen and R. Lobisnki, Pure Appl. Chem., 72 (2000) 1453.
[421] O. Hutzinger, ed. Handbook of Environmental Chemistry, vol. 3, E. Springer, Weinheim, 1990.
[422] R.M. Harrison and D.P.H. Laxen, Environ. Sci. Technol., 12 (1978) 1384.
[423] T. Nielsen, H. Egsgaard, E. Larsen and G. Schroll, Anal. Chim. Acta, 124 (1981) 1.
[424] World Health Organisation, Environmental Health Criteria 3: Lead ,WHO, Geneva, 1977.
[425] P. Grandjean and T.Nielsen, Residue Rev., 72 (1979) 97.
[426] M. Radojevic and R.M. Harrison, Sci. Total. Environ., 59 (1987) 157.
[427] P.T.S. Wong, Y.K. Chau and P.L. Luxon, Nature, 253 (1975) 263.
[428] W.P. Ridley, L.J. Didikes and J.M. Wood, Science, 197 (1977) 329.
[429] R.M. Harrison and D.P.H. Laxen, Nature, 275 (1978) 738.

[430] C.N. Hewitt, S.J.de Mora and R.M. Harrison, Mar. Chem., 15 (1984) 189.
[431] D.P.H. Laxen and R.M. Harrison, Anal. Chem., 53 (1981) 345.
[432] B. Kinsella and R.L. Willix, Anal. Chem., 54 (1982) 2614.
[433] G. Scarponi, G. Capodagliv, P. Lescon, B. Cosma and R. Frache, Anal. Chim. Acta, 135 (1982) 263.
[434] P. Cescon, G. Sacrponi and I. Maret, Sci.Total Environ., 37 (1984) 95.
[435] L. Mart, Fresenius'J. Anal. Chem., 296 (1979) 350.
[436] M. Hiraide, T. Ueda and A. Mizuike, Anal. Chim. Acta, 227 (1989) 421.
[437] S. Tillekeratne, Tmiwa and A. Mizuike, Mikrochim. Acta, 3 (1985) 289.
[438] Y.K. Chau, P.T.S. Wong, G.A. Bengert and O. Kramar, Anal. Chem., 51 (1979) 186.
[439] M. Radojevic and R.M. Harrison, Environ. Technol. Lett., 6 (1985) 129.
[440] C.N. Hewitt, P.J. Metcalfe and R.A. Street, Water Res., 25 (1991) 91.
[441] B. Glad and K. Irgum, Anal. Chim. Acta, 298 (1994) 151.
[442] F. Rapin, A. Tessier, P.G.C. Campbell and R. Carignan, Environ. Sci. Technol., 20 (1986) 836.
[443] G.E. Batley, ed. Trace Element Speciation: Analytical Methods and Problems, CRC, Boca Raton, 1989.
[444] K. Anderson, C.A. Nilsson and O. Nygren, Scand. J. Work Environ. Health, 10 (1984) 51.
[445] O. Nygren and C.A. Nilsson, J. Anal. Atom. Spectrom., 2 (1987) 805.
[446] O. Nygren, Appl. Organomet. Chem., 8 (1994) 601.
[447] R. VanCleuvenbergen, W. Dirkx, P. Quevauviller and F. Adams, Int. J. Environ. Anal. Chem., 47 (1992) 21.
[448] G. Weber, J. Trace Elem. Electrolytes Health Dis., 2 (1988) 61.
[449] Y.K. Chau, P.T.S. Wong, G.A. Bengert and J.L. Dunn, Anal. Chem., 56 (1984) 271.
[450] D.S. Forsyth and W.D. Marshall, Environ. Sci. Technol., 20 (1986) 1033.
[451] G.E. Batley, ed. Trace Element Speciation: Analytical Methods and Problems, CRC, Boca Raton, 1989.
[452] A. Tessier, P.G.C. Campbell and M. Bisson, Anal. Chem., 51 (1979) 844.
[453] G. Whalley and A. Grant, Anal. Chim. Acta, 291 (1994) 287.
[454] M. Blaszkewicz, G. Naumhoer and B. Neidhart, Fresenius'J. Anal. Chem., 317 (1984) 221.
[455] M. Blaszkewicz, G. Naumhoer and B. Neidhart, Fresenius'J. Anal. Chem., 325 (1986) 129.
[456] M. Blaszkewicz, G. Naumhoer and B.Neidhart, Int. J. Environ. Anal. Chem., 28 (1987) 207.
[457] J.D. Messman and T.C. Rains, Anal.Chem., 53 (1983) 1632.
[458] A.M. Naghmush, L. Pyrzynska and M. Trojanowicz, Talanta, 42 (1995) 851.
[459] L. Brown, S.J. Haswell, M.M. Rhead, P. O'Neil and K.C.C. Bancroft, Analyst, 108 (1983) 1511.
[460] G. Weber, J. Trace Elem. Electrolytes Health Dis., 2 (1988) 61.
[461] E.J. Parks, F.E. Brinckman, K.L. Jewett, W.R. Blair and C.S. Weiss, Appl. Organomet. Chem., 2 (1988) 441.
[462] J. Bettmer and K. Cammann, Appl .Organomet. Chem., 8 (1994) 615.
[463] J.S. Blais and W.D. Marshall, J. Anal. Atom. Spectrom., 4 (1989) 271.
[464] J.S. Blais and W.D. Marshall, J. Anal. Atom. Spectrom., 4 (1989) 641.
[465] J. Szpunar, C. Witte, R. Lobinski and F. Adams, Fresenius'J. Anal. Chem., 351 (1995) 351.
[466] K. Sutton, R.C. Sutton and J.A. Caruso, J. Chromatogr. A., 789 (1997) 85.
[467] R. Lobinski, I.R. Pereiro, H. Chassaigne, A. Wasik and J. Szpunar, J. Anal. Atom. Spectrom., 13 (1998) 859.

[468] A. Al-Rashdan, D. Heitkemper and J.A. Caruso, J. Chromatogr. Sci., 29 (1991) 98.
[469] S.C.K .Shum, H.M. Pang and R.S. Houk, Anal. Chem., 64 (1992) 2444.
[470] J. Szpunar, P. Pellerin, A. Makarov, T .Doco, P. Williams, B. Medina and R. Lobinski, J. Anal. Atom. Spectrom., 13 (1998) 749.
[471] D.S. Forsyth and J.R. Iyengar, Appl. Organomet. Chem., 3 (1989) 211.
[472] O. Nygren and C.A. Nilsson, J. Anal. Atom. Spectrom., 2 (1987) 805.
[473] R. Lobinski and F.C. Adams, Anal. Chim. Acta, 262 (1992) 285
[474] R. Lobinski, C.F. Boutron, J.P. Candelone, S. Hong and J. Szpunar-Lobinski, F.C. Adams, Anal. Chem., 65 (1993) 2510.
[475] R. Lobinski and F.C. Adams, J. Anal. Atom. Spectrom., 7 (1992) 987.
[476] J. Szpunar-Lobinski, M. Ceulemans, W. Dirkx, C. Witte, R. Lobinski and F.C. Adams, Mikrochim. Acta, 113 (1994) 287.
[477] C.Witte, J. Szpunar-Lobinski, R. Lobinski and F.C. Adams, Appl. Organmet. Chem., 8 (1994) 621.
[478] R. Lobinski, C.F. Boutron, J.P. Dandelone, S. Hong, J. Szpunar-Lobinski and F.C. Adams., Environ. Sci. Technol., 28 (1994) 1459.
[479] R. Lobinski, C.F. Boutron, J.P. Dandelone, S. Hong, J. Szpunar-Lobinski and F.C. Adams, Environ. Sci. Technol., 28 (1994) 1467.
[480] R. Lobinski, J. Szpunar-Lobisnki, F.C. Adams, P.L. Teissedre and J.C. Cabanis, J. A. O. A. C .Intern., 76 (1993) 1262.
[481] P.L. Teissedre, R. Lobinski, M.T. Cabanis, J. Szpunar, Lobinski, J.C. Cabanis and F.C. Adams, Sci. Total Environ., 153 (1994) 247.
[482] J.I.G. Alonso, J.R.Encinar, I.L. Granadillo, M.M. Bayon, R.G.Fernández and A. Sanz-Medel, Advances in Mass Spectrometry, 15 (2001) 265.
[483] M. Heisterkamp and F.C. Adams, J. Anal. Atom. Spectrom., 14 (1999) 1037.
[484] M. Heisterkamp and F.C. Adams, Fresenius'J. Anal. Chem., 362 (1998) 489.
[485] I.R. Pereiro and R. Lobinski, J. Anal. Atom. Spectrom., 12 (1997) 1381.
[486] T. Nielsen, H. Egsgaard, E. Larsem and G. Schroll, Anal. Chim. Acta, 124 (1981) 1.
[487] C. Nerin and B. Pons, Appl. Organomet. Chem., 8 (1994) 607.
[488] C. Nerin and B. Pons, 2nd International Symposium on Speciation of Elements in Toxicology and in Environmental and Biological Sciences, Loen, Norway, june 15-18, 1994.
[489] J.R. Baena, S. Cardenas, M. Gallego and M. Valcarcel, Anal. Chem., 72 (2000) 1510.
[490] N.P. Vela and J.A. Caruso, J. Biochem. Biophys. M., 43 (2000) 45.
[491] P.L. Teissedre, R. Lobisnki, M.T. Cabanis, J. Szpunar-Lobisnki and J.R. Cabanis, J. A. O. A. C. Intern., 76 (1993) 1262.

This page intentionally left blank

Index