Muhammad Sajid Hamid Akash
Kanwal Rehman *Editors*

Drug Stability and Chemical Kinetics

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ISBN 978-981-15-6425-3 ISBN 978-981-15-6426-0 (eBook)
https://doi.org/10.1007/978-981-15-6426-0

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This book is dedicated
to
Our Beloved and Adorable Little Twinkles
Muhammad Aqdas Akash
&
Zainab Akash

Preface

Stability studies are essential for certifying safety, efficacy and quality of drug products to assess their shelf life. These studies assure that pharmaceutical products will exhibit constant efficacy under specified storage conditions. Developments in pharmaceutical industry show novel ways for preserving the quality of pharmaceutical preparations. Stability is vital for protection, value and quality of a drug. The investigation of drug stability is essential to improve quality, safety and efficacy. Drug toxicity and its adverse effects can be prevented by evaluating parameters related to stability. Upon storage, pharmaceutical products are vulnerable to chemical and physical degradation. These degradations alter pharmacological properties of a drug resulting in reduced benefits and increased toxicity. Physical factors that influence the stability of pharmaceuticals are heat, ionic strength, acid-base catalysis, solvent, light and radiations, oxygen, particle size distribution and moisture.

Advanced kinetic models are used to calculate the degradation rate of biological products, such as protein and virus-based vaccines and emulsion-based adjuvant vaccines. Statistical tools are used to select an optimal number based on variable parameters and analyse experimental data obtained from various steps of kinetic models.

This book "Drug Stability and Chemical Kinetics" provides an introduction to the principles of pharmaceutical analysis in drug stability and chemical kinetics, proposes guidelines for drug stability and stability testing, and mentions methods and protocols for drug stability studies and degradation factors of pharmaceutical products, including physical, chemical and microbial degradation as well as role of decomposition, catalysis and catalytic agents. Finally, it explains various kinetic models in drug stability prevention and therapeutic intervention.

This book provides comprehensive and up-to-date information regarding the principles of pharmaceutical analysis in drug stability and chemical kinetics.

There exists an enormous gap in knowledge between the fundamentals of drug stability and the role of chemical kinetics. This book aims to link the gap existing between drug stability as well as its factors and chemical kinetics as well as its factors. Therefore, this book provides better understanding about each vital component of drug stability and chemical kinetics.

By implication, chapters of this book mention the emerging areas of research in this field and various environmental factors that affect drug quality by physical or

chemical degradation. The book also discusses types and methods of stability tests and storage conditions as well as ICH and WHO guidelines.

This book includes in-depth assessments about various analytical methods and protocols for drug stability studies and factors involved in drug degradation by influencing its kinetic profile. We assure this book will inspire and show innovative paths of research to increase knowledge, awareness and responsiveness about drug stability studies.

Faisalabad, Pakistan — Muhammad Sajid Hamid Akash
Faisalabad, Pakistan — Kanwal Rehman

Acknowledgements

We express our sincere thanks to chapter authors who supported and cooperated a lot. This book is not possible without all their efforts. We wholeheartedly acknowledge the contribution of each author that was essential for this accomplishment.

The support and technical contribution of Higher Education Commission (HEC) of Pakistan is highly significant. Without its provision and funding, it would have been impossible to complete this research. The credit for the accomplishment of this work goes to the research grants (21-667/SRGP/R&D/HEC/2016, 21-1061/SRGP/R&D/HEC/2016, 5661/Punjab/RPU/R&D/HEC/2016, 6429/Punjab/NRPU/R&D/HEC/2016 and 8365/Punjab/RPU/R&D/HEC/2017) sanctioned by HEC. We are highly indebted to HEC as this funding was the key for the success of this project.

Last but not least, we thank Springer Publishers for providing us the platform to publish this book and contribute essential information in this field.

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About the Editors

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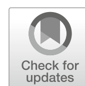
Kanwal Rehman is working as Assistant Professor at Department of Pharmacy in University of Agriculture, Faisalabad, Pakistan. She received her PhD in pharmacology and toxicology from Zhejiang University, Hangzhou, China, where she has done some excellent work by exploring the effect of arsenic compounds and their molecular mechanisms in arsenic-induced carcinogenic and anticancer effects. She displays a keen interest in the pathogenesis of metabolic disorders such as diabetes mellitus and obesity, and works on new therapeutic modalities for the treatment of these disorders. Her other notable work includes the detection of environmental pollutants and their cytotoxic effects. She has published nearly 90 articles with cumulative impact factor of 240. She has also published 40 book chapters and 3 books with international publishers. She is working on three research projects funded by Higher Education Commission (HEC) of Pakistan. Currently, she is investigating the role of pharmacogenomics based upon gene polymorphism on various molecular and metabolic pathways involved in mitogenic disorders, and

focusing on treatment strategies like natural biogenic compounds against different risk factors of metabolic disorders such as pancreatitis, cardiometabolic disorders and hormonal imbalance. Dr. Kanwal Rehman has also been awarded Research Productivity Award by Pakistan Council for Science and Technology (PCST), Pakistan.



1 Principles of Pharmaceutical Analysis in Drug Stability and Chemical Kinetics

Kanwal Irshad, Muhammad Sajid Hamid Akash, Kanwal Rehman, and Imran Imran

Abstract

Pharmaceutical analysis as a separate discipline plays an important role in identification and quantification of ingredients in pharmaceutical formulations. The advancement in pharmaceuticals is helpful for maintaining the quality of pharmaceutical preparations. Stability is a major issue for safety, efficacy, and quality of drug. The stability studies have main objective to estimate shelf life and storage conditions. There are different kinds of stability such as physical, chemical, and microbiological. Various environmental factors such as light, oxygen, moisture, temperature, and pH have influence on the stability of drug substances. The basic principle of pharmaceutical analysis is to ensure that products are free from impurities or within the specified limits. For this purpose, many chemical kinetic methods and instrumental techniques have been developed. This review highlights how various environmental factors affect the quality of drug by physical or chemical degradation pathways.

Keywords

Analytical techniques · Drug stability · Physical degradation · Chemical degradation · Kinetic reactions · Quality of drugs · Shelf life

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_1

1.1 Introduction

The branch of practical chemistry which deals with identification, detection, quantification, qualification, and purification of a substance, separation of constituents of a compound, and identification of structure of chemical compounds is called pharmaceutical analysis. The substance which is to be analyzed may be a single isolated compound or mixture of different compounds or may be present in any dosage form. The pharmaceutical products contain those substances which may be originated from natural sources such as animals, plants, microorganisms, and minerals or synthetic sources [1]. In a comprehensive way, pharmaceutical analysis can be defined as it is an application of the processes that are involved in order to determine the identity of a drug (single or compound) either in its bulk form or pharmaceutical dosage form. To analyze the pharmaceutical substances, various testing have to be performed such as physical, chemical, and microbiological testing [2].

Pharmaceutical analysis can be categorized into two types: qualitative analysis and quantitative analysis. Qualitative analysis is a type of analysis in which the presence of components or impurities is determined which may be expected in a compound or substance. Quantitative analysis is a type of analysis in which the quantity of a drug or substance present in bulk form or in a pharmaceutical formulation is determined. The quality of pharmaceutical substance can be judged by using the correct analytical method. The method should be able to identify the drug in the bulk. The method has capability to determine the stated contents of a drug in the formulation within acceptable limit. It describes the stability of drug content in the formulation and shelf life (the period in which product retains its physical, chemical, microbial, and therapeutic characteristics). It helps to determine the dissolution rate and bioavailability of drug in the body. It should be able to ensure that quality and quantity of drug in the formulation meet with official standards [3].

The pharmaceutical analysis is performed to determine the stability of drug. The stability of pharmaceutical products can be defined as ability of the product to retain its physical, chemical, and microbiological properties and efficacy throughout its shelf life in a container [4]. Mostly, the shelf life of pharmaceutical products is approximately from 3 to 5 years. The drug should maintain its concentration within specified limit and not reduce more than 95% of its original value [5]. Pharmacists are responsible for determining the impact of chemical degradation on the pharmaceuticals. For preparation of sterile products and proper storage conditions of the pharmaceuticals and for determining the shelf life, it is necessary to have knowledge of chemical kinetics. Chemical kinetics deals with the series of reactions involved in the chemical degradation with respect to time [6]. Automatic kinetic methods are used for analysis of many pharmaceuticals because they have significantly increased the quality of testing and reduced the timing [7].

1.2 Drug Stability

The stability analysis of pharmaceutical products is a very complicated process that requires large amount of money, consumption of time, and high level of scientific expertise. The stability analysis has been performed to maintain the quality, efficacy, potency, and safety of the drug. In order to maintain the stability of drugs, care must be taken during the developmental process. The main steps involved in the development of the drug products are pharmaceutical analysis and stability studies of the drug products in order to maintain its quality and efficacy [8].

Stability of pharmaceutical can be defined as the capability of pharmaceutical products to retain its physical, chemical, microbiological, and therapeutic characteristics within the limits as specified by monograph or official standard books during the period of shelf life [9]. Stability studies are performed to evaluate the impact of environmental factors on the formulation, drug substance, or pharmaceuticals. Stability testing helps to determine the shelf life and storage conditions of the products and instructions for labeling on the containers of the products. The stability testing is also very important to generate the data for approval of drug formulation from regulatory authority [8].

1.3 General Objectives of Drug Stability

The basic purpose of stability testing is to determine how the quality of the drug product changes with the passage of time under the different environmental conditions such as humidity, temperature, and light and also to establish the re-test period of the drug products, determine shelf life of the drug products, and determine the storage conditions of the drug products [10]. The stability programs include the study of factors that influence the quality of the products, such as interactions of ingredients with excipients, packing material, and container closure system. The interaction of two or more ingredients is also studied in fixed-dose combination. The stability studies provide information about shelf life, storage conditions, and compatibility of different ingredients in dosage form. The guidelines for stability testing are provided by WHO [11].

1.4 Types of Drug Stability

There are different types of stability such as physical, chemical, microbiological, toxicological, and therapeutic stability. The different types of stability and conditions which are necessary for maintaining the quality of drug are tabulated in comprehensive form in Table 1.1. The stability of drug product significantly depends upon environmental conditions and particular dosage form of drug products. The different dosage form of the pharmaceutical products is given in Fig. 1.1.

Table 1.1 Types of stability and conditions

Stability types	Conditions maintained throughout the shelf life
Chemical stability	Chemical integrity and labelled potency retained
Physical stability	Organoleptic properties remain unchanged
Microbiological stability	Sterility/resistance to microbial growth
Therapeutic stability	Therapeutic effects of the drug product should remain unchanged
Toxicology stability	There is no significant increase in the toxicity of the drug product

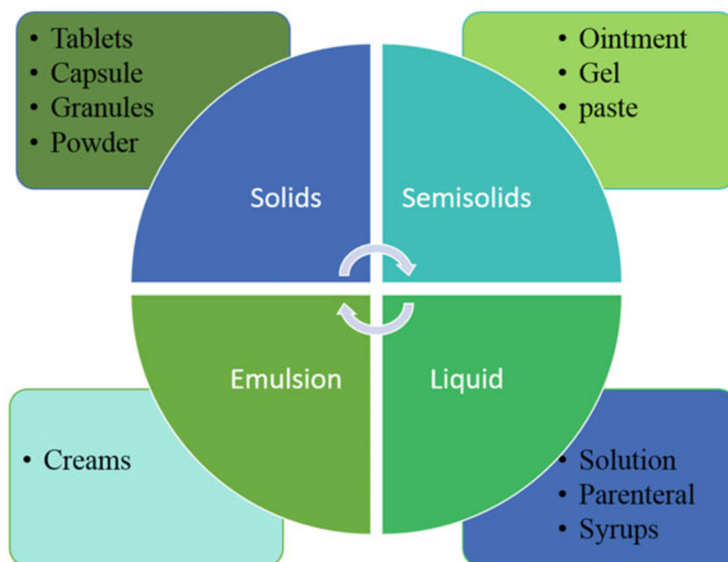


Fig. 1.1 Types of dosage forms of pharmaceutical products

1.4.1 Physical Stability

In physical stability, the physical states are significantly considered for the stability of drug products. The physical properties of drug such as appearance (size, shape, and color), uniformity, palatability, and dissolving and suspending ability should be maintained throughout the period of its shelf life. The physical changes depend upon the physical characteristics of the drug products such as particle size, texture, melting point, polymorphic behavior, and morphology. For liquid dosage forms, the physical stability of the drug products depends upon physical properties such as appearance, discoloration, polymorphism, changes in viscosity, adsorption of drug, precipitation, and growth of microorganisms. These changes in the physical characteristics can cause destabilization of the drug products.

1.4.2 Chemical Stability

All the active ingredients present in the dosage form have the ability to retain their chemical integrity and potency within the specified limit that is labelled on the container or specified in standard books. Drug products have diverse structure and undergo many different degradation pathways. These pathways may include dehydration, oxidation, photodegradation, isomerization, hydrolysis elimination, and complicated interactions with excipients [12] as shown in Table 1.1.

1.4.3 Microbiological Stability

The products should have the ability to retain their sterility/resistance for the growth of microorganism during its shelf life according to the specified limitations. Antimicrobial agents present in the products should retain their activity. Many drug products contain preservatives for protection of drug substances from spoilage. Because contaminated drug products have severe effects on the consumers, that's why the activity of the preservative should be retained during its shelf life. The biological activity of the preservatives must be assessed [13]. Contamination of pharmaceuticals is a critical issue, especially moisture-containing and polymer-containing dosage, because they are the major source for growth of microorganisms. To protect our pharmaceutical product from contamination, we must follow good manufacturing procedures [14]. There are different sources of microbial contamination, which are given in Table 1.2.

1.5 Analytical Techniques

In the pharmaceutics, for development of drug substances, it is necessary to conduct analytical investigation of raw materials, bulk drug substances, intermediate products, finished drug products, degraded products, and many biological samples having drug and their metabolites. For conducting the pharmaceutical analysis, many analytical techniques are used such as titrimetric analysis, chromatography, electrophoresis, spectrometry, high-performance liquid chromatography (HPLC),

Table 1.2 Sources of microbial contamination

Water	Gram-negative groups such as *Pseudomonas*, *Achromobacter*, *Xanthomonas*, and *Flavobacterium*
Air	Mold spores: *Aspergillus*, *Penicillium*, *Mucor* Bacterial spores: yeasts, *Bacillus* spp.
Starches	Coliforms
Gums	*Actinomyces*
Animal products	Coliforms, *Salmonella*
Earths	Anaerobic spore formers: *Clostridium* spp.

and many other electroanalytical techniques. These techniques are also mentioned in the compendial monographs for characterization of quality of bulk drug substances by specifying the range of active ingredients' content in drug formulation [15].

1.6 Kinetic Methods of Analysis

These methods have been developed in 1950 for analysis of pharmaceuticals. These methods have been developed due to advancement in the principles, progress in automated instrumentation techniques, understanding of chemistry, methods of data analysis, and application in analytics. Use of kinetic approach for analysis of pharmaceuticals has many advantages as compared to traditional equilibrium approach [15]. Kinetic methods are used for detecting and measuring the change in the concentration of reactants via signal change with the passage of time when sample and reagents are mixed mechanically or manually. In pharmaceuticals, various techniques such as fixed-time and initial rate methods are frequently used for determination of drug in the formulation [16].

In kinetic methods, different automated techniques are commonly used such as stopped-flow system and continuous addition of reagent (CAR) technique which are based upon open system [15]. Some of drugs are estimated by CAR technique by using photometric technique [17] and fluorometric technique [18]. Catalyst is used to accelerate the chemical reaction and it is feasible for equilibrium state and rate of a reaction. The micellar media can be used in kinetic model to increase the reaction via micellar catalysis. This has significance to improve sensitivity and selectivity and lessen the time for analysis of analyte [19].

In pharmaceutical analysis, multicomponent kinetic estimations are also widely used in analysis of pharmaceutical formulation. The multicomponent kinetic estimations are also referred to as differential rate methods [20]. There are also two approaches, H-point standard addition method and kinetic wavelength-pair method, used for dealing with overlapping spectrum of different components in the binary mixture [15].

1.7 Protocol for Stability Studies

The protocols for stability studies include the following key points:

1. Information on the tested batches, including the chemical formula
2. Composition of the dosage form
3. Packing of finished drug products
4. Literature
5. Specifications of finished drug products
6. Analytical methods for stability study
7. Schedule for stability testing
8. Tabulated test result with the specified limitations

9. Analysis of data
10. Determination of shelf life
11. Commitment for post approval

1.8 Routes of Drug Degradation

The drug degradation can follow physical or chemical pathways depending upon the factors.

1.8.1 Physical Degradation

Many ingredients present in the dosage form exist in different microscopic physical state such as hydrates, solvates, and crystalline states. Many excipients change their state to convert into more stabilized form. The rate of reaction depends upon potential, free energy difference, and energy barrier. Examples of drugs that undergo physical degradation by different pathways are given in Table 1.3.

1.8.2 Crystallization of Amorphous Drugs

Pharmaceutical dosage form is formulated in such a way that poorly water-soluble drug is present in its amorphous state. Because a substance is more soluble in the amorphous phase than crystalline state and crystalline substances have lower free energy as compared to amorphous state, amorphous substances have the ability to undergo a chemical reaction to achieve a more thermodynamically stable state. For example, amorphous nifedipine, under high humidity conditions, is coprecipitated with polyvinylpyrrolidone due to partial crystallization. As a result, changes in the solubility and dissolution may occur.

Table 1.3 Examples of drugs that undergo physical degradation

Physical route of drug degradation	Examples of drugs
Crystallization of amorphous drugs	Oxyphenbutazone, furosemide
Transitions in crystalline states	Raclopride, nitrofurantoin, phenobarbital
Moisture adsorption	Aspirin
Vaporizing	Nitroglycerin
Formation and growth of crystals	Caffeine anhydride, ethenzamides

1.8.3 Transitions in Crystalline States

There are various crystalline forms of a drug called as polymorphs. The various forms of polymorphs have different potential and free energy at different temperature. The transition between different polymorphs occurs depending upon the temperature and humidity. The solubility and dissolution of the drug substance change due to transition in polymorphic states. For example, literature shows that existence of two polymorphic forms of benoxaprofen and three forms of bromovalerylurea has been observed at different temperature.

1.8.4 Moisture Adsorption

Mostly moisture is adsorbed on the surface of solid pharmaceuticals that has significant effect on the physical stability of the drug and excipients such as appearance, dissolution, and solubility. For example, if hydrophilic excipients are added in the aspirin, then it can adsorb the moisture from the environment.

1.8.5 Vaporizing

Many components present in the pharmaceuticals can easily sublime as a result of change in the activity of the substances. For example, nitroglycerine, present in sublingual tablets, has ability to vaporize during storage. The vaporizing ability of nitroglycerin is inhibited by the addition of water-soluble and non-volatile fixing agent, polyethylene glycol.

1.8.6 Formation and Growth of Crystals

Crystals present in the pharmaceuticals are not considered static. Crystals may increase or decrease in size by travelling across the medium. The medium may be a liquid or gaseous phase through which molecules can sublime. Some ingredients may recrystallize or sublime from pharmaceutical dosage forms such as tablets or granules. The crystallization is increased at high temperature in porous tablets. For example, crystallized formation has been observed in the ethenzamide tablets which is confirmed by apparent zero-order kinetics. Crystallization of ethenzamide is enhanced at low and high humidity [21].

1.8.7 Chemical Routes of Drug Degradation

Most of the drugs may undergo degradation by different chemical reactions. Examples of drugs that undergo chemical degradation by different pathways are given in Table 1.4.

Table 1.4 Examples of drugs that undergo chemical degradation

Chemical route of degradation	Drug examples
Hydrolysis	Aspirin, pilocarpine, barbiturates, spironolactone
Oxidation	Morphine, clozapine, phenothiazine, dimercaprol
Photolysis	Furosemide, thiocolchicosides, triamterene, acetazolamide
Dehydration	Theophylline hydrates, ampicillin trihydrates
Racemization	Chlorthalidone, epinephrine, ergotamine, tetracycline

1.8.8 Solvolysis

Due to the presence of a solvent, drugs undergo degradation due to a chemical reaction with the solvent. The most commonly used solvent is water, but a co-solvent may be used in pharmaceuticals. The solvents may act as a nucleophile and have ability to attack on the nucleophile. The most commonly used drugs which undergo attack mostly contain carbonyl compounds. For example, the drugs containing beta-lactam ring are more susceptible to hydrolysis than its linear analogue.

1.8.9 Oxidation

Oxidation reactions play a very important role in the degradation pathway of the drugs. Many drugs undergo autoxidation reaction which occurs due to presence of free atmospheric oxygen called free radical reaction. To protect our pharmaceutical product, there is a need to control the concentration of oxygen in the aqueous solution. Sensitivity of the drug product to oxygen is determined by high oxygen tension. The mechanism by which oxidation takes place involves different pathways such as initiation, propagation, and termination. Oxidation reactions may also be catalyzed by the acid and base. For example, polyethylene glycol suppository base contains hydroperoxides, which are responsible for oxidation of codeine into codeine-N-oxide.

1.8.10 Photolysis

Most of the drugs undergo degradation due to the presence of light. The molecules absorb energy from radiation and undergo photolytic reaction. If this energy is enough to cause the activation of reaction, then degradation of molecule is possible. The molecules containing π electron absorb the wavelength of visible region and as a result degrade. Mostly drugs degrade by absorbing the radiation of wavelength below 280 nm and above 400 nm. But pharmaceutical products must be protected by suitable packing from photodegradation. For example, sodium nitroprusside, administered intravenously to manage hypertension, in the presence of aqueous solution undergoes photodegradation.

1.8.11 Dehydration

Removal of water from a molecule is called dehydration. The molecule undergoes dehydration; as a result, double bond is formed and participates in the electronic resonance with the neighboring atoms. For example, tetracycline and prostaglandin E_2 undergo dehydration by eliminating a molecule of water from its structure.

1.8.12 Racemization

Racemization must be considered in the drug development process. Different kinds of enantiomers of a pharmaceutical compound are significantly different from each other by means of absorption, distribution, metabolism, and elimination. For example, a pilocarpine converts into carbanion by racemization process which is further stabilized by enolate ion. Pilocarpine is also degraded due to hydrolysis of lactone group.

1.8.13 Incompatibilities

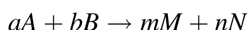
A pharmaceutical dosage form contains active ingredient and number of excipients. A chemical incompatibility may occur in active ingredients and adjuvants. For example, in intravenous admixture, inactivation of cationic aminoglycosides occurs due to presence of anionic penicillin. Glycation of lysine vasopressin may occur due to presence of reducing sugar in aqueous and non-aqueous solvent [22].

1.9 Chemical Kinetics

It deals with chemical reaction rate. Chemical kinetics is very helpful for the identification of mechanism by which drug degrades and how to stabilize it.

1.9.1 Rate of Order of Reactions

The rate of a reaction is defined as the amount of reactant which is lost per unit time or concentration of a degraded product. The reaction rate is explained by a rate equation. For example, a reaction is given below:



$$aA + bB \rightarrow mM + nN$$

where A and B are reactants and M and N are products. Stoichiometric constants are a, b, m, and n, which express the number of moles that take part in the chemical reaction.

1.9.2 Expression of Rate Equation

The rate equation can be expressed as a decrease in the concentration of reactant and an increase in the formation of the product:

$$dC_A/dt = dC_B/dt = -k\ C^a{}_A\ C^b{}_B$$

where k is constant and negative sign indicates that there is decrease in the concentration of the reactant.

1.9.3 Order of Reaction

It is the sum of exponents of the concentration terms in the rate expression. The order of a reaction can be defined mathematically as:

$$n = a + b$$

A reaction is said to be second-order reaction if the value of a and b is 1, assumed to be first-order reaction in relation to a and also considered as first-order reaction in relation to b. Value of a or b may be fraction or integers.

1.9.4 Simple Order Kinetics

Simple order reactions include zero, first, and second order of a reaction. These reactions are considered to be important for pharmaceutical stability studies. For stability study, their mathematical presentations are encountered.

1.9.5 Zero Order of Reaction

In this reaction, the rate of a reaction is not depending upon the concentration of the reactants. The rate at which the concentration of reactant and product changes is constant in zero-order reaction. Many decomposition reactions occur in the solid state or in the suspension form may undergo zero-order reaction. The reaction rate is depending upon many factors such as interfacial surface area and absorption of light. The rate equation of zero-order reaction is expressed as:

$$-dA/dt = k_0$$

OR

$$-dA = dt\ k_0$$

where k_0 is a zero-order constant.

The concentration of A decreases with respect to time as a reaction proceeds:

$$[A]_t = [A]_0 - kt$$

where $[A]_t$ is concentration of A at time t and $[A]_0$ represents the concentration of A at time 0 [23].

1.9.6 First-Order Reaction

The rate of a reaction for first order is directly proportional to concentration of one reactant. Decomposition reactions of many ingredients present in the solid phase and in the suspension follow the first-order reaction. In this order of reaction, concentration of the reactant decreases exponentially with time, and rate of reaction progressively slows down:

$$\text{Rate} = -d[A]/dt = [A]k$$

The concentration time profile of the species that are involved in a first-order reaction follows an exponential decay to limiting value, but products follow an exponential increase to a different limiting value:

$$[A]_t = [A]_0 - \exp(-kt)$$
$$[C]_t = [A]_0[1 - \exp(-kt)]$$

The half-life of a reaction can be defined as the time required for a 50% decrease in the concentration of the reactant from its initial concentration and expressed as $t_{1/2.}$ Similarly if concentration of reactants decreases to 95 and 90%, then half-life for that reactions is expressed as t_{95} and t_{90}. These quantities can be determined by using these equations, if rate constant is known:

$$t_{1/2} = \ln 2/\text{k}$$
$$t_{95} = \ln 0.95/\text{k}$$
$$t_{90} = \ln 0.9/\text{k}$$

For first order of reaction, the time required to lose 50% of drug from initial concentration is the same as concentration of drug is dropped from 50% remaining to 25%, from 25% remaining to 12.5%, and so on.

1.9.7 Second-Order Reaction

In second order of reaction, two reactants are involved in a reaction. In pharmaceutics, first order of a reaction is mostly observed, but in reality, they are second-order reaction. In these reactions, one reactant is in large excess; that's why a change in the concentration of that reactant is negligible and assumed to be first order of reaction. The equation for a second order of reaction is given below:

Table 1.5 Expression of rate equations and concentration time profile for zero, first, and second order of reaction

Order of a reaction	Expression for rate equation	Expression for concentration time profile
Zero	$-dC/dt = k_0$	$[A]_t = [A]_0 - kt$
First	$-d[A]/dt = [A]k$	$[A]_t = [A]_0 - \exp(-kt)$
Second	$-d[B]/dt = k[A][B]$	$1/[A]_t - 1/[A]_0 = kt$

$$A + B \rightarrow C$$

The rate equation for this reaction is expressed as:

$$\text{Rate} = -d[A]/dt = -d[B]/dt = k[A][B]$$

The rate of a reaction is a second order of a reaction but considered to be first order in relation to each reactant.

The concentration time profile for a second order of a reaction can be expressed as:

$$1/[A]_0 - [B]_0\left[\ln [A]_t/[B]_t - \ln [A]_0/[B]_0\right] = kt$$

When, in a reaction, concentration of reactants A and B is the same, then concentration time profile can be expressed as:

$$1/[A]_t - 1/[A]_0 = kt$$

A graph is plotted between fraction remained and time to present the concentration time profile for a zero-, first-, and second-order profile. The rate equations and half-life for a simple reaction are shown in Table 1.5. For plotting concentration-time graph, rate constant is kept the same for all cases, and concentration of initial reactant is also kept identical for a graph and table [24] (Fig. 1.2).

1.9.8 Complex Reactions

In the complex reactions, more than one step is involved. The kinetics of the reaction is estimated by rate equations which may be zero, first, and second order ultimately depending upon the scheme of reactions and rate constant magnitude. Some complex reactions are encountered whose schemes are discussed next.

1.9.9 Reversible Reactions

All chemical reactions are mostly reversible. For reversable rection, equilibrium constant is so large, but overall reaction is treated as virtually one-directional. A simple reversible reaction is shown below:

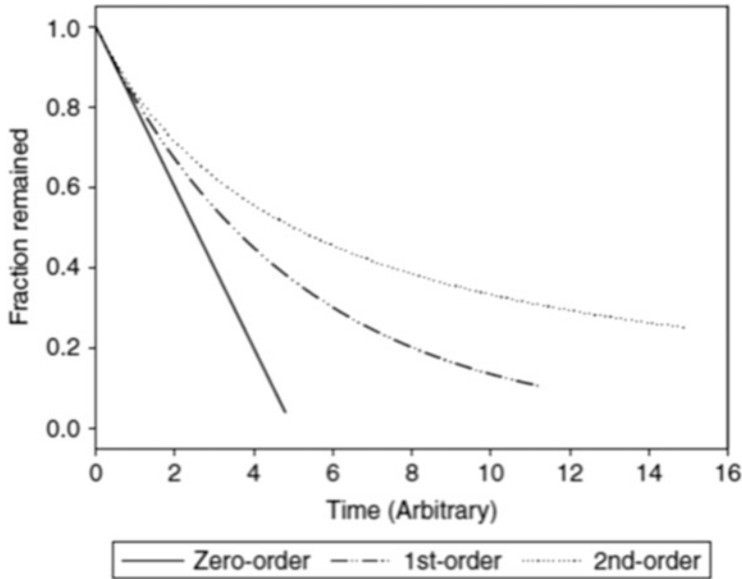
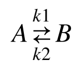


Fig. 1.2 A graph presenting concentration time profile for zero, first, and second order of a reaction. (Adapted from Ref. [24])



$$A \underset{k2}{\overset{k1}{\rightleftarrows}} B$$

where $k1$ is first-order constant for forward reaction and $k2$ is the second-order rate constants for the reversible reaction. The rate equation of a reaction can be written as:

$$\text{Rate} = -d[A]/dt = d[B]/dt = k\ 1[A] - k_{-1}[B]$$

At the equilibrium stage, rate of forward reaction becomes equal to rate of reversible reaction. Then, the concentration of A and B does not change because forward and reversible reactions become equal:

$$\text{Rate} = -d[A]/dt = d[B]/dt = k\ 1[A]_{eq} - k_{-1}[B]_{eq} = 0$$

The eq in subscript indicates quantities of A and B species at equilibrium.

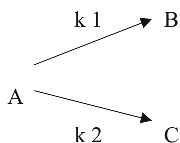
The equilibrium constant for this reaction can be expressed as:

$$K = [B]_{eq}/[A]_{eq} = k\ 1/k_{-1}$$

where K is equilibrium constant, ratio of rate of forward reaction, and rate of the reversible reaction.

1.9.10 Parallel Reactions

A drug can be degraded by more than one pathway involved in the reaction. As a result of degradation of drug, degraded products are formed. The reaction pathways may lead to identical or different degradants. Example for a parallel reaction is shown below:



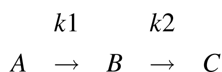
Here $k1$ and $k2$ are the first-order rate constants for reactions $A \rightarrow B$ and $A \rightarrow C$, respectively. The corresponding rate equation is:

$$\text{Rate} = -d[A]/dt = k\ 1[A] + k\ 2[A] = (k1 + k2)[A] = k_{\text{obs}}[A]$$

Here k_{obs} is $k1 + k2$, which is the observed apparent first-order rate constant.

1.9.11 Consecutive Reactions

It is the reaction in which an intermediate product is formed from the initial reactant which is further converted into the final product. The example for a consecutive reaction is given below:



In this simple first-order reaction, A is converted into C through an intermediate B.

The rate equation for this reaction can be written as:

$$-d[A]/dt = k\ 1[A]$$

or

$$d[B]/dt = k\ 1[A] - k\ 2[B]$$

or

$$d[C]/dt = k\ 2[B]$$

1.10 Factors Affecting the Stability of Drug

1.10.1 Temperature

Temperature has a significant effect on overall reactivity of a chemical reaction. The rate of a chemical reaction is enhanced by increasing temperature. In pharmaceutical analysis, for the stability studies of drug, regulatory authorities recommended a high temperature and extreme stress conditions. Arrhenius equation indicates the quantitative relationship between a reaction rate and temperature. According to estimation, the rate of a chemical reaction is doubled by rising 10 °C in temperature, and the reaction occurs rapidly, and the time of a reaction is decreased by a factor of 2.

1.10.2 Light

Light has the influence on the stability of drug via energy or thermal effect, which causes the oxidation of substances present in the drug formulation.

1.10.3 pH

pH has a significant influence on the decomposition of drug. Mostly drugs are stable at pH between 4 and 8. Solubility and degradation of weekly basic and acidic drugs are increased when they are in ionized state. To enhance the solubility of drug, pH of a drug solution is adjusted, but it may be possible that it may lead to instability of a drug substance. To resolve this issue, a water-soluble solvent is added into the solution; as a result, stability of drug increases by reducing the ionization, pH requirement to achieve high solubility, increasing the solubility, and decreasing the activity of water by minimizing the polarity of solvent. For example, 20% propylene glycol is added in chlordiazepoxide injection to enhance the solubility by increasing the stability and ionization of drug. The chemical reactions are catalyzed by pH; the rate of such chemical reaction is monitored by determining the rate of degradation of drug against concentration constant of solvent, temperature, pH, and ionic strength.

Some buffers such as lactate, acetate, phosphate, citrate, and ascorbate buffers are used to minimize the effect of change in pH on drug. Tenfold reaction rate constant is increased by only a change in 1 unit pH. So, before the formulation of a solution in which drug is to be dissolved, it is necessary to develop pH decomposition profile. Data obtained from this profile will help to formulate a solution which is physiologically stable.

1.10.4 Concentration

The solutions having the same drug with different concentration show the same rate of degradation of drug. The ratio of amount of degraded drug and total amount of drug in concentrated solution is less than that of diluted solution.

1.10.5 Moisture

Due to presence of water, microbial growth occurs. Many drugs are degraded due to oxidation, reduction, and hydrolysis. Moisture will promote these chemical reactions; as a result, degraded products are formed.

1.10.6 Water

Water is responsible for hydrolysis of many drugs. Hydrolysis means splitting of substance in the presence of water. Most of drug substances having functional group such as amide and esters undergo degradation. For example, sodium acetate on hydrolysis produces acetic acid and hydroxide. The drugs containing beta-lactam ring undergo fast decomposition reaction than drugs containing ester, imide, and amide groups following first order of reaction. Mostly these types of reactions occur in the presence of divalent metal ions, light, heat, and large concentration of drug [25].

1.11 Conclusion

The main purpose of pharmaceutical analysis is formulation of drug free from any impurities or degraded products to protect human being from any harm. The pharmaceutical formulations must be physically, chemically, microbiologically, and therapeutically stable. The main aim of this review is to use analytical instrumental techniques and kinetic reactions for determining the stability of the drugs. This review highlights the routes of degradation of drugs. Various factors affect the stability of drugs such as oxygen, pH, concentration, temperature, humidity, and moisture.

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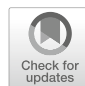

2 Guidelines for Drug Stability and Stability Testing

Kamran Haider, Muhammad Sajid Hamid Akash, Amna Faheem, and Kanwal Rehman

Abstract

Stability studies are pivotal necessities for ensuring safety, potency, and quality of drug products throughout their shelf-life. These guarantee that pharmaceutical products will remain stable and effective under the recommended storage conditions and are considered as prerequisite for approval of any pharmaceutical product. Stability tests are routine procedures that are conducted under different conditions for investigating the effect of variation in temperature, humidity, light intensity, and time on pharmaceutical products. Hence, these studies should be conducted and evaluated according to guidelines issued by ICH (International Conference on Harmonization) or WHO. In this chapter, we have discussed the types and methods of stability tests, storage conditions, as well as ICH and WHO guidelines.

Keywords

Stability testing methods · Guidelines for drug stability · ICH guidelines · WHO guidelines

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_2

2.1 Introduction

Stability studies are crucial in the development of pharmaceutical products as these ensure the quality, safety, and efficacy of drug product as expected by patients [1]. These guarantee the capability of products in maintaining their physical, chemical, and toxicological specifications, which are mainly responsible for their commercial success [2]. These stability studies are pivotal considerations in the development of novel pharmaceutical product or new formulations [3], as these provide evidence in support of safety, potency, and integrity of drug product during the course of shelf-life [4]. These stability studies are also helpful in evaluating the design of manufacturing process, proper storage conditions, as well as selection of appropriate packaging materials. These further assist in predicting the shelf-life of drug product [4–6].

The purpose of these stability studies is to outline the information necessary for registration of drug product [7, 8]. Subsequently, these stability studies also involve product-related factors that can alter the quality such as packaging material, interactions of active pharmaceutical ingredients (APIs) with excipients, as well as container closure system. Also, interaction can be between two or more APIs in case of fixed-dosage combinations [1, 9]. Similarly, regulatory authorities ask the pharmaceutical manufacturers to provide stability data related to active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs) for market authorization and also to follow these recommendations [9, 10]. Equally, the general acceptable range for API is between 95 and 105% during the period available in market, and shelf-life is usually in 1–5 years range for FPP, and after this, these are considered expired [10, 11].

Furthermore, stability studies are primary criteria for ensuring quality as well as approval of manufactured product, being key element for manufacturing trade, along with assigning indications, safety, and potency of drug product [12, 13]. So, stability studies should be performed on all batches of product as well as evaluate all the aspects such as performing analytical studies along with quality check from synthesis till marketing. Consequently, obtained data should be satisfactory till its shelf-life as these are important in mediating their assigned medical activity [11, 14, 15].

2.2 Stability Testing Methods

Stability tests are routine procedures which are carried out during the product development and clarify that either the suitable product will retain their potential effect throughout the recommended life cycle [16, 17]. Besides, accelerated stability studies are performed at initial stages to identify the degradation product, which may appear on long-term storage. The primary reason for conducting and evaluating stability test methods is to guarantee that the product will retain its quality/fitness even in marketplace till its administration to patients [18]. These are classified into the following four categories.

2.2.1 Real-Time Stability Test

These real-time stability tests are mostly conducted for identifying significant product degradation under the recommended storage conditions and performed for longer period of time, depending upon the stability of pharmaceutical product. Also, data is obtained at adjustable frequency and properly monitored for pointing out any instability at specific time period [14, 19].

2.2.2 Accelerated Stability Tests

These studies involve exposure of test product to stress conditions such as elevated temperature, and ultimately then the amount of heat utilized in product failure is determined. This information is further used for predicting shelf-life of product and leads toward shortening of product development period [3]. In addition to elevated temperature, other stress conditions involved in this study are pH, light, agitations, and moisture. These accelerated stability studies demand shorter duration of time in comparison to real-time stability studies. Admittedly, relative accurate stability tests are obtained for the proteinaceous and thermolabile components when temperature is not exceeded from denaturation point [12, 19].

2.2.3 Retained Sample Stability Test

This is a general practice for all products which are marketed and whom stability data is necessary. Typically, this is carried out by choosing one batch, yet two batches are selected when the number of samples is more than 50. Then samples are evaluated at constant time intervals, so this is also known as constant interval method. This sampling method is relatively more realistic as it evaluates product in actual marketplace [2, 20].

2.2.4 Cyclic Temperature Stress Testing

This testing method is not commonly used for market products and designed in accordance to the knowledge of a product, to mimic the corresponding conditions in marketplace storage [3, 21].

2.3 Stability Studies and Their Types

The types of stability studies have major significance in ensuring good-quality products and their ultimate therapeutic response. These are generally classified as (i) physical stability, (ii) chemical stability, and (iii) microbiological stability.

2.3.1 Physical Stability Studies

The physical stability of pharmaceutical products includes their physical appearance, identity, color, odor, and specific gravity along with optical rotation of API [22] [23]. Thus, evaluating the physical stability of the pharmaceutical products is quite necessary for ensuring their integrity and quality [24, 25]. As physical instability demonstrates dysregulation in the physical characteristics of formulated product, this subsequently depends upon the exposed humidity and temperature [22, 24].

2.3.2 Chemical Stability Studies

Chemical stability is the crucial attribute and directly interlinked with the safety, efficacy, potency, and purity of pharmaceutical product [26]. Among the degradation pathways, oxidation and photolysis and hydrolysis are common. Photolytic degradation pathways can be attenuated by minimizing or removing the light exposure, by suitable coating, or by proper closure system. Yet, other pathways include hydrolysis and then followed by the oxidation [27]. Surely, further strategies should be considered for infuriating the undesirable pathways such as minimizing hydrolysis, regulating moisture along with microenvironment pH, or incorporating desiccant in container closure system. Similarly, antioxidant may be added for safeguarding oxidation-related damage [26].

2.3.3 Microbiological Stability Testing

The microbial count may mediate significantly unfavorable effects and can lead to altered safety and integrity of pharmaceutical products. These microbes may be exposed from environmental sources or processing equipment [28, 29]. When a product is contaminated upon exposure, then this gives rise to abnormalities that can lead to alterations, which can be mild (such as discoloration and texture change) to severe (such as toxicity) [30]. Truly, for reducing or complete removal of abnormalities provoked by microbes, strategies such as regulating the water activity of product as well as selection of suitable preservative should be considered and evaluated [29, 30].

2.4 Storage Conditions

The storage conditions should be carefully considered in accordance to environment in which product is intended to be marketed or as in the manufacturer warehouse [31–33]. The maintenance of these storage conditions as recommended is important; otherwise disturbances may lead toward physicochemical degradation. These dysregulated storage conditions may alter the expected potency during the shelf-life. Moreover, these may cause different interaction among compounds in drug

product owing to elevated temperature or may lead to change in physical state and finally give rise to generation of toxic substances [21, 34, 35]. Surely, normal storage conditions which are recommended by WHO involve storage in dry place and well-ventilated premises at a temperature of 15–25 °C, or it may depend upon the climatic conditions (up to 30 °C), whereas intense light, extraneous odor, and contaminations must be excluded [36].

2.5 ICH Guidelines

In order to ensure optimal potency and maximal stability of pharmaceutical products and their manufacture along with distribution and administration to patients, authorities have stressed the manufacturers regarding the availability of stability data. These guidelines were first issued in 1980 [37]. International Conference on Harmonization (ICH) mainly covers the stability guidelines for product registration. This ICH mutually deals with the regulators as well as industries from the United States, European Union countries, and Japan, concentrating on technical necessities for medicinal products comprising new drugs [38]. These stability studies demonstrate suitable storage conditions as well as potency period on outer cover of packaged product, presenting that drug is safe and effective to use throughout the shelf-life [11, 39].

Additionally, ICH provides guidelines and directions regarding the stability studies to the industries [40, 41]. The factors considered by ICH associated with stability studies involve temperature, pH, humidity, light, as well as presence of oxidizing agent. By accepting these ICH guidelines, stability data in the United States, European Union, and Japan would be collaboratory accepted [3, 42]. Thus, a number of guideline documents were developed by ICH demonstrating the stability information required for novel drug product in the ICH regions. So, new drug product must meet these requirements for registration in ICH regions.

The "Q" guidelines released by ICH are:

1. **Q1A** – involves the stability testing of new drug substances and products, which presents the fundamental protocols for the registration. These guidelines show the number and types of batches, storage conditions, as well as stability of container closures for registration of products [43, 44]. Accordingly, this document also discusses acceptable criteria, analytical methods, appropriate tests, as well as stress testing of pharmaceutical products [45].
2. **Q1B** – photosensitivity testing of new drug substances and products; these guidelines deal with light exposure of new drug substances and products. This clarifies that light exposure has no negative impact on the material [46]. This document testing is carried out on single batch for registration stability studies and is a sequenced approach [40, 46].
3. **Q1C** – stability testing of new dosage form; these are shortest among the other ICH guidelines and describe the major stability guidelines for novel formulation

Table 2.1 Overview of ICH code and guideline title

ICH code	Guideline title
Q1A	Stability testing of new drug substances and products
Q1B	Photostability testing of new drug substances and products
Q1C	Stability testing of new dosage forms
Q1D	Bracketing and matrixing designs for stability testing of drug substances and products
Q1E	Evaluation of stability data
Q5C	Stability testing of biotechnological/biological products

of already existing approved medicines and also depict the conditions under which the minimum stability data can be accepted [3, 37, 47].

4. **Q1D** – these guidelines establish principles for reduced stability testing as well as give examples of bracketing and matrixing design; this saves the manufacturer from a number of unnecessary stability testing. Subsequently, minimum stability data also indicates high risks and may not accurately predict expected shelf-life [48].
5. **Q1E** – evaluation of stability data; this illustrates examples of statistical approaches for the stability data analysis and also information associated with method of evaluation. Likewise, this document also elicits stepwise sequences for the evaluation of stability data for the prediction of expiry date [49]. Equally, this describes the application of pool stability tests and linear regressions coupled with statistical modeling to stability data for the purpose of registration [50–52].
6. **Q5C** – stability testing of biotechnological/biological products, as proteins and polypeptides are relatively less stable in comparison to others; this document provides additional information required for product registration of biotechnological/biological products [53].

Overview of ICH codes and guideline titles are shown in Table 2.1.

2.6 WHO Guidelines

The World Health Organization (WHO) changed the ICH guidelines as these guidelines were limited to specific regions and never deal with extreme environmental conditions, as may be found in many countries. ICH guidelines cover only new drug substances and products but not the others which are already present in WHO umbrella countries [2], while WHO guidelines cover all countries and provide instruction throughout the world. Nevertheless, these guidelines cover both the active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs) [54]. World Health Organization started work on the stability studies in 1988, they make certain changes in ICH in 1996. Also, in the years 2003 and

Table 2.2 Overview of climate, included countries, and long-term testing conditions in climatic zones

Climatic zone	Climate	Countries	Long-term testing conditions
I	Temperate	UK, Russia, USA, Northern Europe	21 °C/45% RH
II	Subtropical and Mediterranean	Japan, Southern Europe	25 °C/60% RH
III	Hot and dry	India, Iraq	30 °C/35% RH
IVa	Hot and humid	Iran, Egypt	30 °C/65% RH
Vb	Hot and very humid	Brazil, Singapore	30 °C/75% RH

2006, guidelines were revised due to alteration in long-term storage conditions for supporting climatic zone IV regions [9, 37].

Evidently, guidelines of WHO cover the climatic zones I–III, IVa, and Vb. Whereas, ICH guidelines cover only climatic zones I and II (Table 2.2). Also, recent advances of WHO guidelines emphasize the stress testing of active pharmaceutical ingredients, as this may be helpful in identifying the possible degradation product as well as underlying molecular mechanisms [5]. Correspondingly, countries which are not specifically mentioned adopt either ICH or WHO guidelines as a basis for evaluating stability performance [45]. Similarly, WHO promotes the usage of advanced analytical techniques that enabled better performance. HPLC-NMR is the most famous technique generally used for the stability testing, although others are also used [5].

2.7 Importance of Stability Studies

The main reason for stability testing of pharmaceutical products is to ensure the expected therapeutic response of drug for its indication, as most of the drugs lose their pharmacological response at a particular time after their development and may change to toxic decomposition products [55]. These stability studies provide important information to sponsors for developing such a product which can resist in hard environmental conditions, as drug may face rigorous environment from the time of its manufacture till its administration [56]. This is important as in some dosage form, extreme environmental and processing conditions such as relative humidity and elevated temperature lead toward instability problems [57].

Decidedly, stability testing also ensures the use of appropriate excipients, as pharmaceutical excipients can mediate significant instabilities, which can lead toward altered attributes of pharmaceutical product and ultimately failure of dosage form [58]. Hence, selection of suitable excipients which have appropriate compatibilities with API and retain desired attributes throughout the shelf-life of a product is crucial for good product development [59]. Therefore, stability testing provides a variety of methods and tools for investigating instabilities along with interaction of pharmaceutical dosage forms, as well as in selecting suitable

excipients during the product development [60]. Consequently, these also urge researchers for development of more stable pharmaceutical products and also developing novel approaches for mitigating the unfavorable instability problems [61].

2.8 Conclusion

Stability studies have a significant importance in ensuring quality of drug products as well as formulations. These studies at developmental stage provide crucial information related to storage conditions, container closure system, and excipients of the pharmaceutical product. The maintenance of these guidelines guarantees the patients about potency, safety, efficacy, and quality of the product throughout their shelf-life. Obviously, stability tests are the routine procedures used for evaluating the maintenance of these guidelines. These stability guidelines are regulated internationally by ICH and WHO. Surely, these regulatory authorities stress the industries for developing good-quality products as well as to fulfil the stability guidelines for manufacturing and marketing a product. Additionally, these stability guidelines and testing procedures urge the researchers for developing more stable, effective, and economical products. Hence, summarizing the above, these guidelines should be developed according to the requirement of regions in which a product is to be manufactured or marketed and also should be strictly followed by manufacturing industries.

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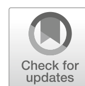

3 Chemical Kinetics and Its Applications in Drug Stability

Mutayyba Fatima, Muhammad Sajid Hamid Akash, Muhammad Fawad Rasool, and Kanwal Rehman

Abstract

The study of chemical kinetics is very crucial as it plays a significant role in drug stability, which has direct impact on storage and release characteristics of drugs. Various kinds of instabilities occur that lead to the degradation of drugs. Drug degradation is of different types including physical, chemical, and biological degradation. This chapter presents an overview of chemical kinetics and a brief introduction of rate of reaction and its types. The following text also provides detailed information about the physical and chemical factors that affect the rate of reactions. The types along with various mechanisms through which degradation occurs are also included in this chapter.

Keywords

Chemical kinetics · Rate of reaction · Drug stability · Drug degradation

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_3

3.1 Introduction

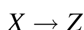
Kinetics deals with rate of reactions, and chemical kinetics includes the study of rate of chemical reactions. It is the rate of conversions of reactants through various mechanisms into final products [1]. Chemical kinetics are described by different mathematical models which then serve as tools for scientists and chemists to control and understand a variety of chemical reactions. In chemical reactions, there is the breakage of bonds present in reactants, followed by the formation of new bonds to form the product [2]. Reactions can occur fast and slow, and chemical kinetics measure this rate that either reactions precede at a slow or faster pace. Other than providing information about the speed of reaction, it also provides detail about the mechanism of reaction including knowledge about each step in the reaction process. Mechanism of reaction is the step-by-step process through which a reaction occurs. A mechanism provides information about all the stages of a chemical reaction [3].

3.2 Rate of Reaction

It is the speed at which the reactants (initial) are converted into products (final). Every reaction has two rates:

1. The rate at which the reactant declines
2. The rate at which the final product appears

If we consider two species X as initial reactant and Z as final product,



$$X \rightarrow Z$$

This reaction can be expressed either as disappearance of X or as appearance of Z. This rate of chemical reaction can be expressed as a change in concentration of reactants in respect of time [4]. As the rate of reaction is related to the concentration of reactant that decreases with respect to time, the dimension must be of concentration which is moles per liter second or moles per liter minute. Rate of reaction for the above equation would be:

$$\text{Rate} = -d(X)/d(t)$$

where d(X) is the speed at which reactants change into products and is negative because reactants decrease.

$$\text{Rate} = +d(Z)/d(t)$$

where $d(Z)$ is the speed at which the product appears and is positive because the concentration of products increases with respect to time.

Mathematical equation that relates concentration of species with that of time is called as rate law or rate equation.

3.2.1 Types of Rate of Reactions

Depending on the velocity, rate of reactions is classified as slow and fast rate of reactions.

3.2.1.1 Fast Reaction Rates

Reactions that occur at a faster pace are fast reaction. Example of fast reactions includes burning [3].

3.2.1.2 Measuring Fast Reactions

Some reactions occur instantaneously, and therefore, special techniques are required in order to measure such reactions. There are two major difficulties that occur in fast reactions: the first is it is difficult to measure initial time accurately and second is the time in which quantity of substance is measured is comparable with half-life of reaction. In order to cope with these complications, flow, pulse, and probe methods are used. The basic principle in pulse and probe method is that a short pulse is directed to a chemical system, which is then followed by a probe, which gives spectroscopic data of what happened after the initial pulse. In flow methods, two different solutions or gases are added in the mixing tube, and then the mixture will flow along the tube. Concentrations of reactants and products are then measured along the tube by different spectroscopic methods which correspond to different reaction times [2].

3.2.1.3 Slow Reaction Rates

Reactions that occur at a low speed have slow reaction rates. Example of reactions that have slow rate is disintegration of a plastic bottle in sunlight [3].

3.2.1.4 Measuring Slow Reactions

To measure slow reactions, the best approach is to alter reaction conditions like an increase in temperature. For instance, if the temperature of a mixture containing hydrogen and carbon is increased up to 500 °C, the reaction will occur rapidly in this condition, and reaction rate can be studied [2].

3.3 Factors That Affect the Reaction Rate

Factors affecting the rate of reaction are classified into physical and chemical factors, which are then further classified as follows:

3.3.1 Physical Factors

3.3.1.1 Surface Area

Surface area is the area of space available for reaction. This term is most significant in solid state of matter. Solids have a definite shape, and therefore limited sites are

available for the atoms or molecules of solids to react with other reactants. However, when solids are crushed into powders, surface area increases and hence the rate of reaction, as more atoms collide with reactant [4].

3.3.1.2 Concentration of Reactants

To predict the rate of chemical reactions, the reaction theory is used according to which reaction occurs when reactant molecules come closer and collide. If the proportion of reactants is more, there will be an increased concentration of reactant particles moving together; hence, more collisions will occur; resultantly, the rate of reaction will be increased [4].

3.3.2 Chemical Factors

3.3.2.1 Temperature

The measurement of average kinetic energy of molecules or atoms in the system is called as temperature. Temperature has impact on all chemical reactions, and depending upon an increase or a decrease of temperature, this change can either be positive or negative. For example, degradation and evaporation of solutions and samples slow down at low temperature, and these phenomena get four times slower while storing solutions and samples at a temperature of 4 °C. For every 10 °C increase in temperature, the reaction rate will be doubled for most of the chemical processes [5]. Temperature is directly proportional to the kinetic energy of molecules or atoms in a system, and therefore, when the temperature of the system is increased, the collisions to overcome the activation energy will also be increased. Activation energy as described by Swedish Scientist Svante Arrhenius in 1889 is the minimum energy required by reactants to initiate a chemical reaction [6]. When the temperature of a system is increased, the following two factors occur:

1. More intense collisions
2. Greater frequency of collisions

As the intensity of collision of molecules within the system increases, the rate of reaction also increases. For example, the rate of reaction of enzymes Maxatase and Alcalase was higher at 45° C and lower at 37° C, when pH of the medium is 8.2. When activation energy is calculated, it appears that the higher the temperature, the higher is the rate of reaction and the lower is the activation energy [7].

3.3.2.2 pH

pH of a medium can have a significant impact on the stability of a drug. More than tenfold change in the reaction rate constant results in only 1 pH unit shift. For calculating optimum pH for stability of drugs in solution, drug versus reaction rate profiles are constructed [8].

3.4 Applications of Chemical Kinetics in Drug Stability

Chemical degradation of pharmaceutical products is a common phenomenon in our everyday lives. Careful storage of non-sterile pharmaceutical products and manufacturing and storage of sterile drugs are some examples that are totally based on knowledge about chemical kinetics of pharmaceutical products. From both economical and safety points of view, it is very crucial for pharmacists to have thorough knowledge about chemical kinetics [9]. Kinetics plays a basic role in the development and evaluation of drug substances by analysis of inhibitory mechanisms. Kinetics is applied in enzymology on routine basis in order to determine the inhibition mechanism and to check the relative efficacy and efficiency of various inhibitors. Chemical kinetics has multiple applications in designing a pharmaceutical product ranging from drug manufacturing characteristics to its action inside the body.

3.4.1 Stability of Drug Products

Rate process is the one that leads to incompatibility and inactivation of drugs which occurs by the breakdown of drug substances into less active or unwanted metabolites. The various ways through which a pharmaceutical drug product can degrade are briefly explained in Sect. 4.5. By proper storage of drug products, it is possible to reduce the rate of these reactions, hence maintaining the stability of drug products throughout their shelf life.

3.4.2 Pharmacokinetics of Drug

It includes absorption, distribution, and elimination of drug through metabolism inside the body. All of these mechanisms involve chemical kinetics.

3.4.3 Dissolution of a Drug

Rate process involves the conversion of solid drug molecules into an aqueous solution of drug substance.

3.4.4 Chemical Kinetics and Drug Stability

For designing any pharmaceutical drug product, the most significant factor is its stability. For a drug to show its pharmacological activity, it is very crucial that it is stable in formulation form until used for the intended purpose. Several forms of instabilities occur that impair the activity of the pharmaceutical drug product, which is the cause of rejection of drug. First of all, chemical decomposition of the drug may

lead to a decrease in the quantity of an active drug present in the pharmaceutical dosage form. Second, during the process of decomposition, a toxic product may form which can cause adverse effect. Moreover, instability of product may lead to reduction in therapeutic efficacy of the drug. Other than this, instability can also affect physical appearance of drugs such as cracking or creaming of emulsions or breaking or mottling of tablets. Such changes may not affect the pharmacological activity, but patients will not compromise over the unpleasant appearance of pharmacological dosage forms. In addition, sometimes the drug substance itself does not degrade, but the excipients in the formulation may get degraded affecting the therapeutic activity of drugs. The following are the drug degradation pathways.

3.4.5 Chemical Degradation Pathways of Pharmaceutical Drug Product

Chemical degradation of a product occurs through the following mechanisms, which have been summarized in Fig. 3.1.

3.4.5.1 Photodegradation

It is the process in which drugs and excipient molecules are degraded by light. Exposure of drugs to a photolytic source, i.e., a source emitting radiation between

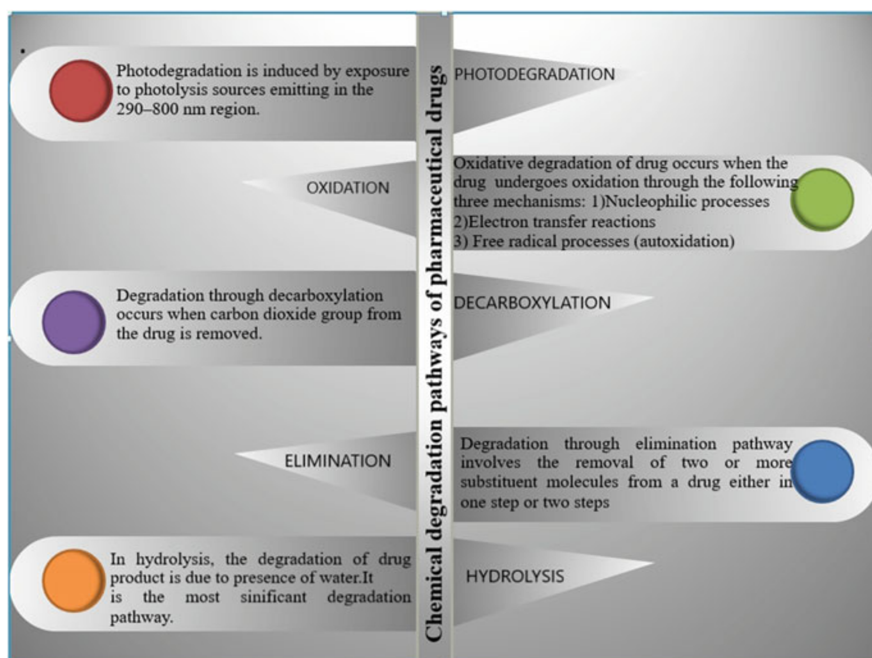


Fig. 3.1 Chemical degradation pathways of pharmaceutical drug product

290 and 800 nm, results in photodegradation. Multiple sources of light emit radiations in this range, for instance, fluorescent lamps, metal halide lamps, sunlight, and some indoor lighting sources. If a molecule absorbs light, its electronic configuration changes to an excited state. Electrons present in the outermost shell are susceptible ones as they are least firmly bounded electrons. Photolysis is a consequence of the absorption of light, or radiation energy, allowing for quantum restrictions by a molecule A, to produce an unstable excited-state species, and the energy can be lost either by a radioactive mechanism in which the energy is given in the form of fluorescence or by a radiation-less mechanism. These mechanisms can be physical or chemical in nature. The physical decay results in the loss of energy in the form of heat or by collision with other molecules [10].

In the pharmaceutical industries, photostability studies of pharmaceutical products are carried out to ensure the safety, potency, and efficacy of formulated drugs during manufacturing, throughout shelf life and upon administration. Photostability studies are an integral part of drug development because the presence of light can affect the drug characteristics if the drug is photosensitive. For example, when the photostability of flunitrazepam was measured, it was investigated that flunitrazepam shows a drop of more than 60% in concentration when stored in light, after 8 h, though no such change was seen in samples that were kept in the dark [11, 12].

3.4.5.2 Oxidation

Oxidative reactions are somewhat common drug degradation pathways. Oxidation is a loss of electrons or an increase in the oxidation state.

$$\text{Reduced form} \leftrightarrow \text{Oxidized form} + \text{ne-}$$

For example, the aqueous solution of ascorbic acid (vitamin C) degrades in aerobic conditions, and the molecule is then oxidized into dehydroascorbic acid [13], as shown in Fig. 3.2.

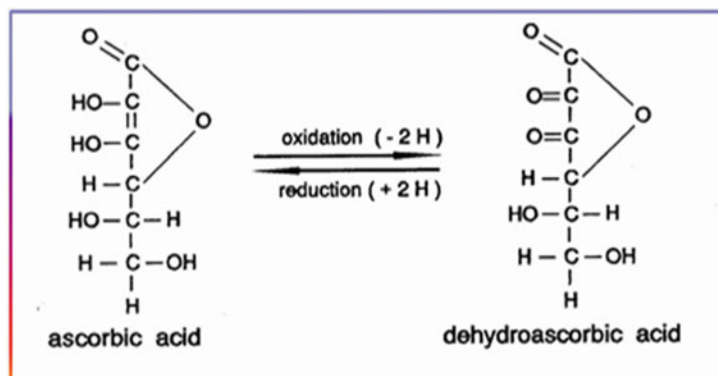


Fig. 3.2 Oxidation of ascorbic acid

After hydrolysis, oxidation is the most significant drug degradation pathway. But because of complex nature of oxidative reactions, despite their significance in stability of drugs, the studies related to them have not been well developed [14]. Organic compounds undergo oxidation mainly through one of these mechanisms:

3.4.5.3 Nucleophilic/Electrophilic Process

Oxidation might be related to nucleophilic displacement reactions as oxidative addition reactions include the attack of a nucleophile on carbon by metal [15]. Oxidative addition reactions and electrophilic reduction reactions are among the most significant transformations in organometallic chemistry [16]. This process mostly occurs between organic reactants and metal peroxides.

3.4.5.4 Electron Transfer Process

This process includes chemical reactions that involve the transfer of electrons. In this type of oxidation, molecule undergoing the oxidation process loses electron; however, the molecule that gains electron undergoes the process of reduction. Basically, electron is transferred from a donor having low affinity to a molecule that is being reduced. This reaction is often catalyzed by metals (transition metals). This process is quite simple; however, this can be made complex by the system in which it occurs [14].

3.4.5.5 Autoxidation

Autoxidation is a complex oxidation mechanism that proceeds through a free radical chain process. It is a common degradation mechanism for unsaturated fats, but a number of drugs containing carbon-carbon double bonds also undergo oxidation. Free radical chain process has three main steps [17]. At first, there is formation of free radical by photochemical or thermal breakdown of an R-H bond. The first step is catalyzed by metal ions such as Ni21, Cu21, and Fe31. In propagation step, molecular oxygen is added to the free radical. In the rate-determining step or RDS, the peroxyl radical extracts the hydrogen atom from R-H bond to produce another R• radical. The rate of rate-determining step depends on how strong the CH bond is which is being breached. Last is the termination step, in which the chain reaction is cleaved when two free radicals react with each other to form non-radical products. Thiols after oxidation can form acid, disulfides, sulfonic acids, and sulfenic acid through various mechanisms such as autoxidation, electron transfer reactions, and nucleophilic processes [14].

3.4.5.6 Elimination

The removal of one or more than one substituent molecules from a drug either in a single step or multiple steps is called elimination. For instance, synthetically made antitumor drug trimelamol (N2,N4,N6-trimethylol-N2,N4,N6-trimethylmelamine) gets degraded when hydroxymethyl groups were removed from its structure. To evaluate the kinetics of this reaction, HPLC was used [18]. Bimolecular reaction or

E2 reaction is one which consists of a single-step mechanism, however unimolecular or E1 reactions have two-step mechanisms.

3.4.5.7 Decarboxylation

Removal of a carbon dioxide group from a compound is called as decarboxylation; it is not a common phenomenon. Drugs containing carboxyl groups in their structure are susceptible to degradation through the process of decarboxylation under some circumstances. Antibiotics containing carbonyl group specifically on the beta carbon of a carboxylate anion or a carboxylic acid undergoes beta-Keto decarboxylation. For instance, this type of decarboxylation occurs in these antibiotics: carbenicillin sodium, ticarcillin sodium, carbenicillin free acid, and ticarcillin free acid. A major example of this reaction is 4-aminosalicylic acid which after decarboxylation, in aqueous medium, is converted into 3-aminophenol, as shown in Fig. 3.3. In alkaline medium, the drug is in ionized form, and therefore this reaction is faster in acidic medium.

Decarboxylation does not have to be a photochemical degradation. Few carboxylic acids, for instance, p-aminosalicylic acid, undergo decarboxylation by loss of CO2 from the carboxyl group. Decarboxylation of p-aminosalicylic acid has been shown to be a rate-controlling proton addition followed by rapid decarboxylation [19].

3.4.5.8 Hydrolysis

Hydrolysis includes degradation of drug product due to presence of water. A large number of drug products are prone to lyses in the presence of water. Esters and amides are the two most prominent functional groups that are susceptible to hydrolysis on storage. The hydrolysis of these groups occurs due to nucleophilic attack on carbonyl carbon resultantly cleaving carbon, nitrogen, and carbon-oxygen bond [20]. Examples of drugs that have ester functional group and undergo hydrolysis are aspirin, cocaine, procaine, etc. Examples of drugs that have amide functional group and undergo hydrolysis are acetaminophen, chloramphenicol, and indomethacin [21].

3.4.6 Physical Degradation of Pharmaceutical Drug Product

Physical degradation of product occurs through the following mechanisms, also shown in Fig. 3.4.

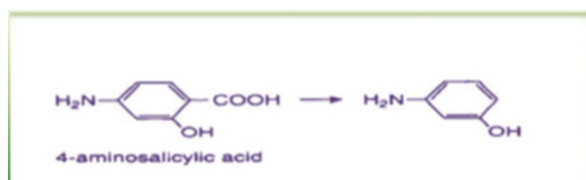


Fig. 3.3 Decarboxylation of 4-aminosalicylic acid into 3-aminophenol

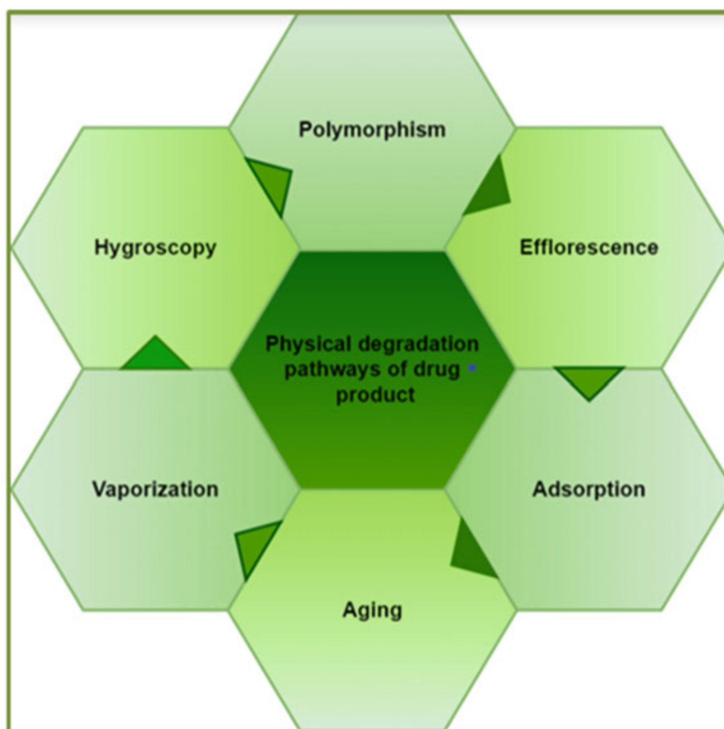


Fig. 3.4 Physical degradation pathways of drug product

3.4.6.1 Polymorphism

The term "polymorphs" is used for different crystalline forms of the same compound, and these forms occur when there is a change in pressure, relative humidity, and temperature. Examples of drugs that show phenomenon of polymorphism include formaldehyde and aminopenicillins [21].

3.4.6.2 Efflorescence

It is a phenomenon in which a drug product loses water, and resultantly the concentration of drug in the product increases. For example, solution loses water and becomes supersaturated [21].

3.4.6.3 Adsorption

While storing drugs, plastic material is mostly used especially in primary packaging and is occasionally used for administration of a drug product. During this period of direct contact of a plastic with the drug product, adsorption of drug may occur resulting in drug loss. For instance, 40% of quinidine gluconate is lost when the drug is administered with a traditional polyvinyl chloride IV administration set [8].

3.4.6.4 Hygroscopy

It is a phenomenon in which drug product absorbs water from environment or surroundings and resultantly the drug deteriorates. For example, powders deteriorate under moist storage conditions [21].

3.4.6.5 Vaporization

Volatile compounds, for instance, camphor, volatile oils, ethers, and ketones, can escape from formulation through the process of vaporization, which results in drug loss affecting the efficacy of the product. Therefore, such products are needed to be placed in a tightly close container with temperature considerations [8].

3.4.6.6 Aging

Aging is the process in which the physical and chemical properties of the active substances and excipients present in the drug change. It is the most interesting phenomenon in physical degradation of drug product but occurs rarely. It affects the dissolution and disintegration characteristics of the dosage form [8]. For example, in aminophylline suppositories, melting time increases from about 20 min to more than 60 min after storing at 22° C for 24 weeks' period. This increase in melting time resultantly decreases the drug bioavailability [22].

3.4.7 Microbiological Degradation of Pharmaceutical Drug Product

Microorganisms are adaptable to a huge range of conditions and therefore possessed a risk for the pharmaceutical drug products. Deterioration of drug product due to the presence of microorganisms can make the product harmful for human use or can have adverse effects on the properties of drug product. Contamination of a product by microorganisms affects both the quality and safety of drug product. A microbial control strategy should have to be employed, and therefore thorough information about the microbe entry points in a process along with potential of microbial contamination of medium, buffers, and drug product is required [23].

3.5 Conclusion

The speed at which reaction occurs is termed as rate of reaction, and the study of the rate of reaction is known as chemical kinetics. Reactions can progress both at a faster and slower pace, and there are multiple factors affecting these rates. Among various chemical and physical factors, the most important factor is temperature; every 10 °C increase in temperature can double the speed of reaction; therefore, temperature should be carefully monitored and maintained according to the product specifications. Chemical kinetics plays a significant role in drug design, development, and manufacturing, to ensure stability of drug. Drug instability leads to drug degradation which ultimately results in loss of drug activity. Drugs can be degraded by microbes and also by physical and chemical pathways. Chemical pathways of

drug degradation are most prevalent, and the majority of drugs degrade through chemical mechanisms.

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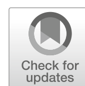

Methods and Protocols for Drug Stability Studies

4

Hammad Ahmed, Waseem Hassan, Ghulam Murtaza, Sahar Bakht, and Furqan Muhammad Iqbal

Abstract

Stability is a significant quality attribute for drug molecules and pharmaceutical preparations. Investigation of drug stability is essential to improve quality, safety and efficacy. The drug toxicity and adverse effects prevented by proper evaluation of parameters are related to stability. The toxic effects could be due to degraded impurities, drug metabolites or functional groups of drug molecules. Therefore, stability studies are planned to identify and maintain the product quality, throughout the shelf life. The major role of such studies is to predict shelf life, determine the suitable storage condition and suggest the label instructions. Stability studies are deemed as prerequisite for the recognition and endorsement of pharmaceuticals. Stability studies should comply the guidelines of the ICH, the WHO or other agencies deemed fit. These guidelines postulate the outline for the execution of stability studies on both drug and dosage form. The aim of these guidelines is not to constrain the experimentation but to execute the proper and meaningful experiments. The scope of these guidelines is limited to pharmaceutical dosage forms and any feed impregnated with medicinal product. Stability studies are necessary for the development and registration of newer drug.

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_4

Keywords

Stability studies · ICH guidelines · WHO guidelines

4.1 Introduction

Stability studies of drugs are carried out to identify the time in which the pharmaceuticals maintain their physical, chemical, microbiological and pharmacokinetic properties and characteristics. Bright light, sunlight, radiations, temperature, humidity and certain other environmental factors cause the drug to degrade. Despite the product is not expired. While developing pharmaceuticals, principal stages include analysis and stability studies. These studies are mandatory for the identification and maintenance of transparency and potency until its expiry date. To maintain the product integrity and suitability, it should be prevented from the exposure of any environmental factor up to a maximum extent. Drug stability studies are also carried out for the newly developed drugs and dosage forms to identify the expiry of the certain dosage forms. Similarly, physically and chemically active substances in the formulation and the nature of container-closures used and the storage conditions [1].

The decomposition procedure and methods of degradability of active substances are usually accessible in the literature along with adequate analytical methods. Therefore, stability studies are generally limited to the dosage form. Shelf life of the product means that any substance reduces to 90% of its original concentration. In pharmaceutical terms, shelf life is used to indicate the stability of the product, and it is denoted as expiry date. Expiration differs for each pharmaceutical preparation.

Besides that, many factors affect the stability of a pharmaceutical product like microbiological changes including the growth of microorganisms and variations in preservative efficacy. Likewise, the statistics obtained while testing is an imperative prerequisite for regulatory approval of any drug formulation or dosage form [2]. The testing of pharmaceutical products is a multifaceted task, and it requires substantial funds, ample time and appropriate scientific proficiency and capability to obtain apposite excellence, effectiveness and harmlessness pharmaceuticals. Technical and viable triumph of any pharmaceutical merchandise lies in the successful product development alongside appropriate stability of the product [3].

There are certain factors that influence the stability of the drug including stability of the active ingredient, physical and chemical interaction among the active ingredients and excipients, particular manufacturing steps, "dosage form, container/closure" used for packaging and "environmental (light, heat and moisture) circumstances" that come across during shipment, post marketing storage and handling. Moreover, oxidization, hydrolysis and reduction are the most likely degradation reactions that occur in the pharmaceutical products. These physical changes are observed when there is fluctuation in the erstwhile explained factors that influence the stability [4].

4.2 Importance of Conducting Drug Stability Studies

These types of studies are patient centred, and patients are undergoing from the particular ailment for which the drug is developed. The degradation of the drug product is converted into harmful chemical, loss of claimed therapeutic effect that might result into catastrophic results including death. For instance, glyceryl trinitrate tablets indicated for angina and cardiac arrest or certain antibiotics can lead to dose dumping. Due to these reasons, firstly, it has been a legal obligation to carry out these stability studies and present the data to the regulatory agencies or supervisory watchdog prior to approval of a new drug. Secondly, the stability studies provide confidence to the manufacturer that the product will retain all its claimed attributes including efficacy and effectivity for as long as it is in the market [5].

4.3 Types of Drug Stabilities

Particular pharmacopoeia "US Pharmacopeia and British Pharmacopoeia" explains the following standards [6, 7].

4.3.1 Physical Stability

This type of stability covers the physical properties like appearance, colour, dissolution, palatability and suspendability. The physical stability is conducted to check uniformity and release rate; henceforth adequate physical stability is essential for the optimal efficacy and safety of the product.

4.3.2 Chemical Stability

In fact, this type of stability is conducted to determine the potential of a drug to resist its degradation due to the chemical reactions triggered by air, atmosphere, temperature, etc.

4.3.3 Microbiological Stability

To identify the presence of potential harmful microbial growth and to attain the desired sterility, such type of studies is conducted.

4.3.4 Therapeutic Stability

This type of stability is conducted to ensure that therapeutic effect remains unchanged.

4.3.5 Toxicological Stability

This type of stability is conducted to check the formation of certain toxic compounds, due to the degradation of the product. This includes photostability testing, forced degradation studies and degradation product studies (flowchart shown in Fig. 4.1). According to the time frame, stability studies [8] are classified in Table 4.1.

4.4 Methods for the Stability Studies of Drugs

Stability testing is a procedure performed for all the pharmaceutical products at various stages of the product development. It is suggested to follow the test protocols stated in the official compendia, because these are officially accepted and extensively

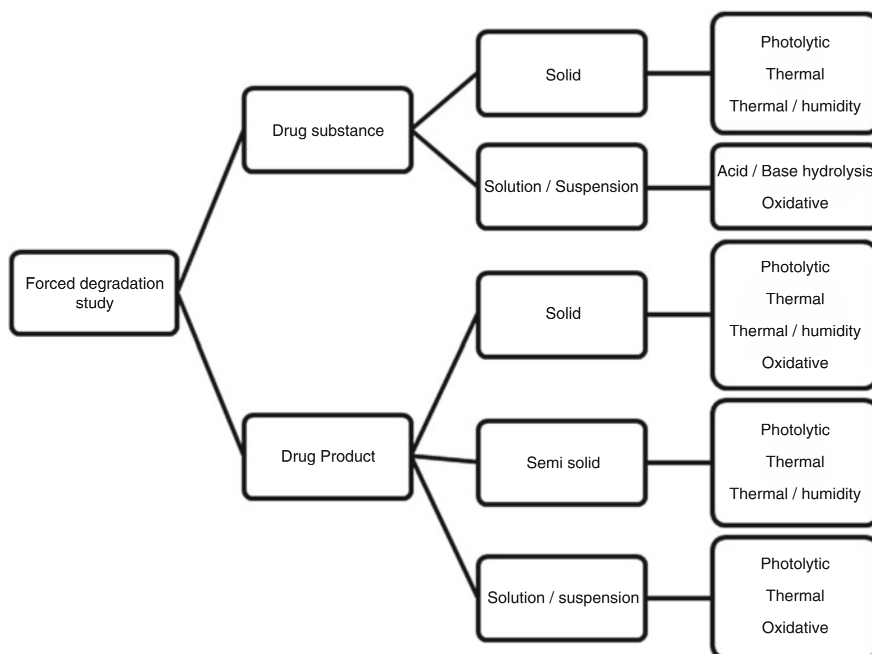

Fig. 4.1 Schematic representation of forced degradation study of both the drug product and drug substance

Table 4.1 Showing the type storage and duration of study

Type of study	Storage conditions	Study period (months)
Long term	25 ± 2 °C and 60 ± 5% RH or 30 ± 2 °C and 65 ± 5% RH	12
Intermediate	30 ± 2 °C and 65 ± 5% RH	06
Accelerated	40 ± 2 °C and 75 ± 5% RH	06

researched and practised, and this is the reason these are ultimately better accepted [9]. However, if any alternate protocol is followed, it must be validated twice. The following methods and procedure are employed for the stability studies.

4.4.1 Real-Time Stability Testing

Real-time stability testing is usually implemented for extended period of time to permit and help to analyse substantial degradation of the drug under the recommended storage conditions. The time period for undergoing test product solely is contingent to the stability of the product. This clearly states that the drug is not decomposed for this time period from inter-assay variation. The testing is usually performed in such a way that the analyst can perform the testing at regular interval and appropriate frequency so that the analyst can distinguish the daily degradation of the drug. The extent of degradation is compared by standard single batch (reference batch), of which stability studies are carried out before or stability attributes have been well known. In this regard, the component materials and the instruments operated should be in the consistency throughout the stability testing. The studies shall not be reproducible if there is a change in both reagents and instruments. In order to change either reagents or instruments, it should be again standardized and optimized [10].

4.4.2 Accelerated Stability Testing

Such type of testing is executed at relatively higher temperatures and that decomposes the product. The data originated from such study is used to assess the shelf life and help to compare the relative stability of alternative formulations. Such type of studies are helpful to predict the shelf life; hence it cuts the duration to identify the stability of the drug. Moreover, along with the temperature, further stress conditions are also applied such as strenuous moisture, light, pH and gravity. As the name suggested, such instability studies are taken place in a very reduced time as compared to real-time testing. The stability projections are carried out at four distinct stress temperatures [11].

The accelerated stability studies are easily calculated by the Arrhenius equation:

$$K = Ae\frac{-Ea}{RT}$$

where:

K = specific rate constant
A = frequency factor or Arrhenius factor
Ea = energy of activation
R = real gas constant 4.184 J/mol.k
T = absolute temperature

In this method, the drugs are placed at different temperatures such as 40 °C, 60 °C, 70 °C, 80 °C, 100 °C, etc. These studies are also carried out at room temperature (25 °C) and at fridge (0–4 °C) temperatures [12]. The specimen is sampled and examined and has undergone stability testing at different time intervals. At first year, the sampling is performed at 3 months' and 6 months' interval the following year and yearly afterward. Frequent sampling and testing is required for the rapidly degraded products. The purpose of increasing the temperature and providing stress to the products is to ascertain the decomposition potential of the substances.

Conferring to the existing International Committee of Harmonization (ICH) guidelines, 40% products must be kept at controlled room temperature. As per ICH and World Health Organization (WHO) guidelines, the storage condition for accelerated stability studies is 40 °C ± 2 ° C; 75% RH ± 5% RH. If the product is unstable on the recommended temperature and humidity, intermediate conditions are used, i.e. 30 °C ± 2 °C; 65% RH ± 5% RH. The Food and Drug Administration (FDA) suggested the sampling testing for 0, 2, 4 and 6 months, respectively. The WHO prescribes for 0, 1, 2, 3, 4 and 6 months. The ICH prescribes the test to be performed for every 3 months in a year, 6 months in 2 years and yearly thereafter. Moreover, these accelerated tests are mainly executed for stability and moisture absorption testing. This test is implemented for the entire pharmaceutical preparations, but primarily this is a test utilized for dispersed systems like pharmaceutical emulsion and suspension testing [13].

4.4.3 Retained Sample Stability Testing

It is a conventional type of testing for each marketed finished product. In this category of testing, the stability is done by randomly selecting one batch for a year. If the number of samples surpasses 50, then sampled from two batches. For the newly marketed batches, every batch should be sampled later on that may reduce from 2% to 5%. Such stability studies are conducted to forecast the shelf life. The probable extreme shelf life of every product is to be 5 years which is predictable to the test samples at 3, 6. 9, 12, 18, 24, 36, 48 and 60 months. This testing method is also known as constant interval method. In fact this type of testing is fundamentally more accurate because it trials the product not only in the idealized retained sample storage conditions but also in the actual marketplace [14].

4.4.4 Cyclic Temperature Stress Testing

This method of testing is not frequently exercised. In this technique, cyclic temperature stress tests are planned, and attempts are made to provide similar type of conditions that the product may face in the market. The sampling and testing are

deliberately carried out by a 24-hour cycle. Based on temperature and storage conditions and predicted by physicochemical degeneration of the drug product, the lowest and extreme temperatures are noted for product. In order to forecast the shelf life, the 20-cycle testing is recommended [15].

4.5 Protocol of Drug Stability Testing

The protocols and the procedures for stability studies are prerequisite intended for initiating and conducting testing. The protocol should be in written documented form that must address mechanisms and key steps for conducting the appropriate stability studies. Furthermore, the protocol is different for novel drug and previously marketed drug [16]. In order to attain accuracy and precision, the analytical procedures and steps should essentially be validated and verified. Specified assay nominated for stability studies should be performed. The recommended tests for related drug products should be validated prior the testing is performed. Likewise, the approval and rejection standards for each analytical result in addition to the presence of degradation products should also be predetermined. Preferably it should be fixed in numerical limits [17]. Optimum stability protocol should address the following parameters.

4.5.1 Number of Batches

The protocol for selecting the number of batches to perform stability studies is the following:

1. Stability testing is carried out in batches as performing the stability studies in a single step is difficult; hence, they are divided into batches.
2. For a stable product, the stability studies are executed on a single batch.
3. For the unstable and newly registered products, studies are carried out on three consecutive batches. If any one of the batch displays instability, then the studies shall be performed for six consecutive batches, and if the instability persists, then all the batches have to be rejected as they cannot pass the stability testing [18].

The preliminary batches are not full-scale production batches. For the first three batches with subsequent approval from the regulatory bodies, long-term studies following the same protocol as in approved drug applications should be performed. The laboratory data are not accepted for the primary stability data. Random sampling technique should be used for the selection of samples from the batches [19].

4.5.2 Containers and Closures

The choice of containers and closures is extremely crucial for the stability studies. The product should be packed in such a container that it not only protects the product from the harmful environment but also made up of a suitable material that could not harm the product itself. Usually the packaging of the drugs is borne in materials like aluminium strip packs, blister packs, Alu-Alu packs, HDFE bottles, etc. Possibly the secondary packaging is present. Testing should be performed on each of the container and closure of the product since the inappropriate shippers and packing materials can degrade the drug. Prototype containers can be used for the bulk packing. The finished goods products must be placed in tested suitable containers because inappropriate containers can contaminate the product; hence shelf life of the product can be affected [20].

4.5.3 Orientation of Storage of Containers

Drug products like solutions and semi-solid should essentially be placed upright, so that the drug comes in contact with the containers. This aids to determine any chemical change occurring in the drug that can lead to the degradation of the drug. Such type of degradation might be due to either absorption or loss of water.

4.5.4 Sampling Time Points

The testing is vital at specific time intervals to ascertain and authenticate the stability of the new drug substance. The testing should be scheduled monthly in the first year, then after 6 months for the second year and then yearly thereafter throughout the predicted shelf life. However, in the case of accelerated stability studies the testing should be conducted in a minimum of three time points, for instance, 0, 3 and 6 months. If the testing should be done for the different batch size and strength of the same product, then retained stability testing method should be followed, i.e. a smaller number of time points. This testing plan is merely depending on the bracketing and matrixing statistical designs. Bracketing is the design only when the samples on the certain design factors such as strength and package size are tested at all the three time points as in full design. The factors that can be matrixed can include the strength, batches, container sizes and intermediate time points [21]. Sampling plan covers the number of samples to be placed in stability chambers. This plan merely depends on the number of sampling points and the product to be required for each test to be performed as depicted in Table 4.2.

Table 4.2 Plan for stability testing of new products

Temperature and relative humidity	Sampling time point (months)	Method
25 °C/60%	3, 6, 9, 12, 18, 24, 36	Long term
30 °C/35%	3, 6, 9, 12, 18, 24, 36	Long term
30 °C/65%	3, 6, 9, 12, 18, 24, 36	Long term
30 °C/75%	3, 6, 9, 12, 18, 24, 36	Long term
40 °C/75%	3, 6, 9, 12, 18, 24, 36	Accelerated

Table 4.3 Stability test storage conditions for drug products

		Storage conditions for			
		ICH		WHO	
Storage	Stability study	Temperature and relative humidity	Time (months)	Temperature and relative humidity	Time (months)
Room temperature	Long term	25 ± 2 °C and 60 ± 5%	12	25 ± 2 °C and 60 ± 5%	12
		or			
		30 ± 2 °C and 65 ± 5%			
	Intermediate	30 ± 2 °C and 65 ± 5%	6	–	–
	Accelerated	40 ± 2 °C and 75 ± 5%	6	–	–
Refrigerator	Long term	5 ± 3 °C and –	16	5 ± 3 °C	–
	Accelerated	25 ± 2 °C and 60 ± 5%	6	–	–
Freezer	Long term	−20 ± 5 °C and –	12	−20 ± 5 °C	–

4.5.5 Test Storage Conditions

The storage conditions are selected solely on the basis of the climatic zones in which the finished product has to be marketed. The WHO and the ICH have designed general recommendation on the storage conditions [21]. The ICH and the WHO explain the storage conditions for drug products, as shown in Table 4.3.

4.5.6 Mean Kinetic Temperature

In accordance to ICH guidelines, "*Mean Kinetic Temperature* is a single calculated temperature, which degrades the same amount of the drug as degraded, by the different temperatures during the particular time period." It is a valuable tool for the stability studies. It aids to analyse the degradation of stability sample. It is predictable from the temperatures of stability chambers [22].

4.5.7 Test Parameters

The test parameters used in the stability studies must be designed to estimate the stability of samples. The test of sample is generally carried out to determine the quality, purity, efficacy and identity. Therefore, appearance, quantitative assay, degradation potential, dissolution and moisture content are benchmark tests for the stability studies. Microbiological tests comprise of sterility, microbial count and preservative measures whenever applicable. Besides that, stability testing parameters also includes the determination of heavy metals, residue of ignition, residual solvents, etc. Depending upon the nature of the product, several other tests are also performed that are discussed in ICH guidance Q6A [23].

4.6 Stability Test Equipment

Stability chamber is used for the stability testing. Stability chamber is the particular environmental compartment intended to maintain the optimal storing environment and approximate the product stability based on real-time, accelerated and long-term protocols. Various sizes of the stability chambers are available. Usually smaller chambers are selected for accelerated testing; however, for long-term testing, the walk-in chambers are ideal. Walk-in chambers are up to the size of rooms. Rooms are designed to ensure the optimum storage condition, uninterrupted for years. They are equipped with appropriate recording, safety and alarm devices. Besides that, photostability chambers are also designed having two types of light sources (near-UV fluorescent and artificial daylight lamps) and can be operated with both modes with and without temperature and humidity control. It is essential to achieve entire exposure of 1.2 million lux hours [5].

4.7 Climate Zones

The stability studies are implemented and followed globally (Table 4.4). Due to the environmental differences, one standard cannot be practicable to conduct the stability studies. For this reason, the ICH has divided the world into five climatic zones depending on their climatic conditions, temperature and humidity levels of the stability chambers, which are adjusted to carry out stability studies [24].

Table 4.4 Classification of climatic zones

Zone	Type of climate	Temperature	Relative humidity
I	Temperate zone	21 °C ± 2 °C	45% ± 5%
II	Mediterranean/subtropical zone	25 °C ± 2 °C	60% ± 5%
III	Hot-dry zone	30 °C ± 2 °C	35% ± 5%
IVa	Hot humid/tropical zone	30 °C ± 2 °C	65% ± 5%
IVb	Hot/higher humidity	30 °C ± 2 °C	75% ± 5%

4.8 Applications of Stability Studies

The main objectives and application of stability testing are explained below.

4.8.1 For Drug Development

Once the formulation and manufacturing process of product have been established, the manufacturer conducted frequent accelerated stability tests that help to predict the product stability. Similarly simultaneous real-time studies essentially be commenced; the aim of such study is to confirm the product stability [25].

4.8.2 For Approval from Regulatory Bodies

In order to register drug in the respective drug regulatory authority, it is essential to support your application with stability studies. Usually the results of both accelerated and real-time studies are submitted in the dossier. When the product needs to be diluted or reconstituted prior to use (e.g. a powder for injection or an oral suspension), stability data essentially should explain optimal storage period and surrounding environment for such dosage forms [6].

4.8.3 Post-Registration Period

In order to validate the tentative expiry date and the storage conditions, the manufacturer should continue on-going real-time stability studies. Other results of on-going stability studies are verified in the course of Good Manufacturing Practices inspections. The government regulatory bodies and watchdog agencies to conform the quality and safety of products carry out follow-up inspection and testing programmes [26].

4.9 Conclusion

The physical, chemical and microbial drug stability is essential for ensuring the quality of pharmaceutical formulations. The objective of stability evaluation is for providing evidence about the integrity of drug product affected under various environmental factors, like temperature, pH, radiations, light and humidity. Due to integrated research and with escalating knowledge and awareness, the regulatory requirements for the pharmaceutical products are increasingly stringent to attain the required objectives. Therefore, the stability tests should confirm the appropriate scientific parameters. It would require understanding and application of up-to-date regulatory prerequisites in accordance with climatic zones.

Conflict of Interest Authors declare no conflict of interest.

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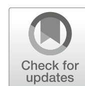

Physical Basis of Degradation of Pharmaceutical Products

5

Tauqeer Hussain Mallhi, Rabia Khokhar, Aisha Khokhar, Syed Nasir Abbas Bukhari, and Yusra Habib Khan

Abstract

Pharmaceutical products are susceptible to chemical and physical degradation upon storage. These degradations give rise to modified pharmacological outcomes resulting in altered therapeutic and toxicity profiles. Therefore, degradation studies are foremost during product development to evaluate the stability of pharmaceutical products. The pharmaceutical products intended for human and animal use must fulfill the requirements of chemical, physical, solid-state, microbial and photo stability. Several external and structural factors transmute the physical and chemical properties of the pharmaceuticals throughout manufacturing and storage. Physical stability secures prime importance while designing any dosage form. Temperature, ionic strength, acid-base catalysis, solvent, light and radiations, oxygen, particle size distribution, and moisture are the basic physical factors that influence the stability of pharmaceuticals. Different dosage forms show distinct effects due to physical instability. Mostly deterioration of dosage forms destroys the physical appearance and efficacy of the product. The stability study of a pharmaceutical product requires a rigorous understanding

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_5

of the nature of incorporated drug and the excipients. The degradation of a drug can generate sorption, crystal growth, crystallization, or even loss of water in a formulation. Similarly, the physical nature of excipients must also be kept under consideration during manufacturing and storage as excipients generally undergo chemical, microbial, and physical instability. The physical instability of excipients widely includes precipitation, crystallization, agglomeration, phase transformation, polymorphism, and solubility changes. To detect the degradation of pharmaceutical products, several chromatographic techniques and high-performance liquid chromatography are employed. However, stability testing methods such as real-time testing, accelerated stability testing, retained sample stability testing, and cyclic temperature stress testing can also be exercised to ensure the optimal stability and efficacy throughout the shelf-life of pharmaceutical products.

Keywords

Degradation of pharmaceutical products · Stability of pharmaceutical products · Chemical kinetics of pharmaceutical products

5.1 Degradation and Stability of Pharmaceutical Products

Pharmaceutical products incorporate multiple drug particles ranging from small molecular size to large polymers. Some drugs, under certain conditions, are prone to chemical deterioration, resulting in the destruction of the molecular structure of the drug. However, fewer drugs are susceptible to physical degradation, primarily affecting the physical state of a pharmaceutical product. Consequently, these degradations may alter the pharmacological characteristics of products, rendering the product less efficacious and more toxic for consumption. The quality of pharmaceuticals must be retained under all phases of manufacturing, storage, and transportation. Therefore, the comprehension of stability and degradation factors of pharmaceutical products is critical [1].

The pharmaceutical products have strong propensity for degradation upon storage. The stability of a pharmaceutical product is defined as the capacity of the dosage form to retain the physical, chemical, therapeutic, and microbial attributes throughout the time of storage and patient use. According to the US Pharmacopeia (USP, 2016), stability is defined as the extent to which a product maintains its similar characteristics throughout the shelf-life as it contains at the time of production. The shelf-life and expiry date of a product are important in determining the stability of pharmaceutical products. A time period during which a pharmaceutical product, upon proper storage, maintains its physical, chemical, and microbial stability within the allowable limits is termed as shelf-life, whereas the date specified on the primary and secondary packaging of the pharmaceutical product indicating the termination of shelf-life is termed as expiry date or expiration date [2].

5.2 Factors Affecting Stability of Pharmaceutical Products

There are numerous factors that alter the physical and chemical properties of a pharmaceutical product. These factors exert an influence on product stability either during manufacturing or storage [3]. Such factors include:

- *External Factors*: Temperature (simvastatin and aspirin), light (nifedipine and vitamin D), pH (clopidogrel and cisplatin), oxidation (ascorbic acid and levodopa), enzymes (glucagon-like peptide-1 and parathyroid hormone).
- *Structural Factors*: Ester pro-drug, lactone acid conversion and tautomerization, chiral interconversion, and acyl glucuronide metabolites [4]

5.3 Types of Stability

Considering a huge number of pharmaceutical products for human and animal consumption, and for better understanding, the stability falls under five important categories:

5.3.1 Chemical Stability

Chemical stability of pharmaceuticals is the absence of degradation of any chemical, i.e., drug, preservatives, or any excipient, that is added in the formulation. The chemical stability of a liquid dosage form is affected by several chemical reactions, i.e., hydrolysis, oxidation, decarboxylation, and others. Certain computerized technologies are being utilized to predict the chemical degradation pattern of pharmaceuticals [5].

5.3.2 Photostability

A significant number of drug products are prone to degradation upon exposure to light in the course of manufacturing and storage. This results in the loss of therapeutic effectiveness and change in the safety profile of the pharmaceutical product. The photodegradation occurs through multiple reactions in different drugs, such as photo-oxidation of ascorbic acid, photo-reduction of riboflavin, and photo-cyclization of meclofenamic acid. However, the photodegradation can be avoided by utilizing suitable packaging material. Amber-colored and opaque containers are appropriate for light-sensitive products.

5.3.3 Physical Stability

Physical stability deals with physical changes in pharmaceutical products. As these modifications are based on the physical factors of the products, therefore, physical stability of solid and liquid dosage differs in nature. The physical degradation of solid dosage forms results in polymerization, desolvation, crystallization, and moisture adsorption. Moreover, physical degradation causes liquid dosage forms to precipitate, discoloration, and microbial contamination.

5.3.4 Solid-State Stability

The solid-state stability concerns with the chemical and physical modifications in a solid dosage form mainly due to temperature and moisture. The modifications involve precipitation, crystallization, particle size growth, and deliquescence.

5.3.5 Microbial Stability

Microbial stability should be established in a pharmaceutical product throughout storage and usage. Suitable preservatives are incorporated in multi-dose preparations to minimize spoilage with the contaminants and microbes [3]. Various types of stability of pharmaceutical preparations along with the mechanism of instability are briefly discussed in Fig. 5.1.

5.4 Physical Basis Affecting Stability of Pharmaceutical Products

The majority of studies related to drug stability have highlighted the chemical stability of pharmaceuticals. Nonetheless, physical stability must also be kept under consideration while designing a dosage form. The physical factors that influence the stability of pharmaceuticals include (a) temperature, (b) ionic strength, (c) acid-base catalysis, (d) solvent, (e) light and radiations, (f) oxygen, (g) particle size distribution, and (h) moisture.

5.4.1 Temperature

The stability of pharmaceuticals is markedly affected by the temperature change. For better stability, a product is often stored in a refrigerator at 2–8 °C. Sometimes a product is even stored in a freezer below −15 °C. However, the freezing temperature renders storage and transportation troublesome for some unstable products. Additionally, a liquid product must be thawed before administration without using any heat source to avoid degradation. Therefore, some drugs are more stable in the

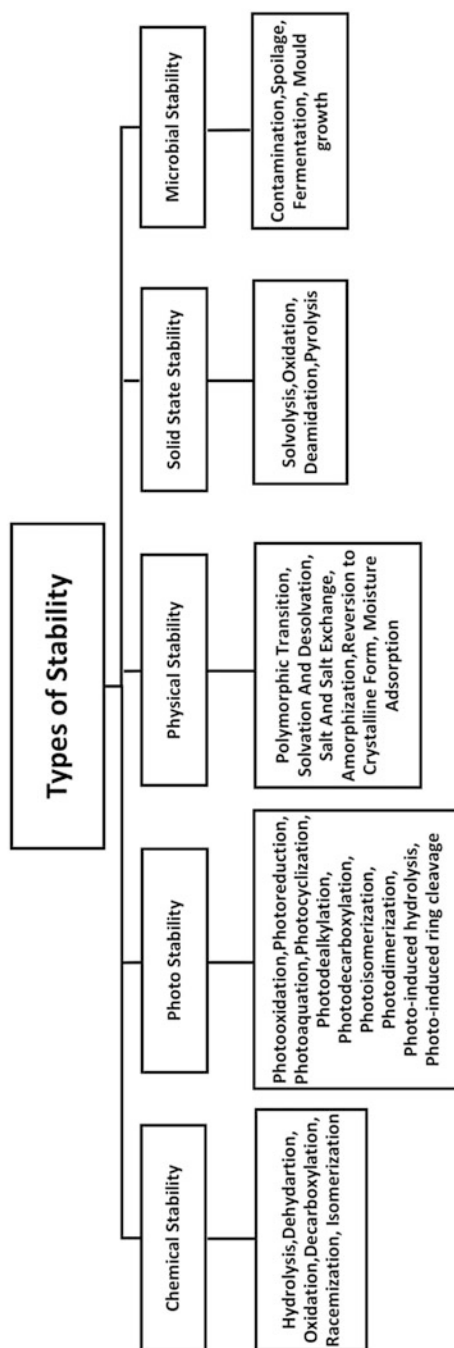

Fig. 5.1 Types of stability of pharmaceutical products with distinct mechanisms of instability

refrigerator than in a freezer, e.g., amoxicillin in solution form [6]. The freezing may initiate deterioration of live vaccines and biopharmaceuticals as well. This can be overcome with the incorporation of some cryoprotectant substance, i.e., trehalose, in the product. However, the temperature changes mainly affect the finished products during storage and shipping [7, 8].

5.4.2 Ionic Strength

Ionic strength is concerned with the number of ionic species in the pharmaceutical product. Fluctuations in the ionic strength with the addition of an electrolyte may affect the deterioration process in a solution. However, these reactions do not produce any marked influence in drug solutions; it is of sheer importance while investigating the effect of pH on degradation rate in laboratory. Therefore, the ionic strength of buffer solutions is closely monitored to avoid any modification in endpoints.

5.4.3 Acid and Base Catalysis (pH)

The pH plays a critical role in determining the stability of an aqueous solution. Thorough investigations are performed at preformulation phase to determine the effect of pH on degradation rate. Several acid-base catalysts influence the pH of a formulation. A catalyst is an entity that escalates the rate of a chemical reaction but does not take part in a reaction itself. A specific acid catalysis and base catalysis is catalysis by hydrogen ions and hydroxyl ions, respectively. For better comprehension of acid-base catalysis, a graph is plotted between first-order rate constant and pH. An inverted bell-shaped graph obtained thus yields important details regarding the degradation process. The degradation rate is higher at both low and high pH due to specific acid-catalyzed and base-catalyzed hydrolysis, respectively. However, a flat area is obtained in the center, which indicates the non-catalyzed hydrolysis region. This is specifically a region within which the pH of a product must be maintained to ensure its stability. Cephalosporins, mainly cefuroxime, follow this shape of the graph. However, in case of penicillins, a V-shaped graph is obtained indicating a narrow region of uncatalyzed hydrolysis and thus narrow optimal stability range. In such cases, pH is sharply maintained within the ideal limit.

Several drugs experience ionization up to a level that it starts relying on the pH of the formulation. The pH affects the extent of ionized drug, thereby changing the rate of degradation reactions. For example, aspirin exhibits this character of ionization. There are several species other than H_3O^+ (acid) or OH^- (base) which may speed up a degradation process. It is known as general acid and general base catalysis. Buffer ions are the main reason of such catalysis in a formulation. Hydrolysis of chloramphenicol catalysis by phosphate and acetate buffers is an example of buffer ion catalysis.

5.4.4 Solvent

A solvent in a pharmaceutical product is chosen not only considering its safety and compatibility but also the dielectric constant. The dielectric constants deal with the polarity of the solvent. The higher dielectric constant indicates a greater polar nature of solvents. In a liquid dosage form, a polar solvent can be replaced with the nonpolar solvent to avoid degradation through hydrolysis. However, the solid dosage forms are comparatively firm than the liquid formulations. Tablets may encounter reactions if the water is adsorbed on their surface. Aspirin tablets if not stored properly may undergo hydrolysis that yields pungent smell of acetic acid from the tablets. Some injections are stored in freeze-dried powder form, such as penicillins, and are reconstituted with normal saline or water prior to administration. Suspensions are considerably stable than solution as drug is suspended in the insoluble medium.

5.4.5 Light

Light and radiations may interfere with the stability of a product resulting in photodegradation of the drug substance. The use of suitable containers may protect the formulation from light exposure. Tinted or amber-colored glass containers are useful to lessen the penetration of UV rays. Additionally, an opaque secondary packing may also serve to further minimize the exposure of formulation to light.

5.4.6 Oxygen

Among all the degradation processes, the oxidation reactions are least affected by the temperature changes. Therefore, decreasing temperature may be less suitable to achieve stability in this case. For this purpose, the containers are often rinsed with an inert gas, i.e., nitrogen, to lessen the quantity of oxygen in the formulation. In spite of this, the formulation will not be oxygen-free completely. However, this is widely practiced during manufacturing for single-dose ampoules.

Oxidation reactions are usually influenced by the change in pH. High pH accelerates the oxidation reactions. Therefore, aqueous preparations that are vulnerable to oxidation must be manufactured at low pH. Cu^{2+} and Fe^{3+} ions generally present in pharmaceutical products accelerate oxidation at multiple stages. To overcome this, chelating agents are generally incorporated that bind with the metal ions and neutralize them. Ethylenediaminetetraacetic acid (EDTA) and citric acid are the most commonly used chelating agents that stabilize the product. Additionally, antioxidants are also used in the formulation. Antioxidants perform its role through multiple mechanisms. They either remove the oxygen behaving as oxygen scavengers or by terminating free radicals. Sodium metabisulphite and ascorbic acid are the most commonly used water-soluble antioxidants, whereas ascorbyl

palmitate, butylated hydroxytoluene, and α-tocopherol are the most commonly used lipid-soluble antioxidants [2].

5.4.7 Particle Size Distribution

Particle size distribution is considered important primarily in case of suspensions. The size of the drug particles affects the bioavailability and dissolution profile of several drugs. Drugs with larger particle size exhibit less solubility and dissolution and eventually decreased bioavailability. Particle size is highly notable in certain drugs in which absorption solely depends upon the dissolution. For example, in case of spironolactone, griseofulvin, and nitrofurantoin, the absorption is dependent upon the particle size of drug molecules [9]. As stated by Stokes' law, the particle size also influences the content uniformity as larger particles sediment rapidly in comparison to the smaller particles [10].

Keeping suspensions under consideration, the particle size is also affected by crystallization, sedimentation, and agglomeration. Crystallization mainly results because of Ostwald ripening, modification of polymorphic forms, and temperature changes [11].

5.4.8 Moisture

The moisture usually adsorbs on the surface of solid dosage forms when stored improperly. It causes alterations in the appearance and dissolution rate of the formulation rendering the product physically unstable. The moisture adsorption depends on the physical properties of drugs as well as an excipient. For example, if hydrophilic excipients are incorporated with aspirin, the moisture adsorption immensely increases. For determination of the kinetics of moisture adsorption, Zografi and co-workers developed an equation considering water-soluble substances as:

$$W' = (C + F) \ln \frac{RH_i}{RH_0}$$

where W' is moisture rate constant, RH_i is relative humidity, RH_o is critical relative humidity, C is a conductive coefficient, and F is radiant coefficient [12]. Moreover, a better understanding of physical stability along with the chemical stability is of prime importance while compounding extemporaneous suspensions. The instability of tacrolimus, a potent immunosuppressant, may result in diminished bioavailability consequently leading to tissue rejection and even death [13].

5.5 Possible Effects of Physical Instability

Different formulations show distinct effects due to physical instability. Mostly deterioration of dosage forms results in the destruction of the physical appearance and efficacy of the product. Other possible complications include sorption, evaporation, and contamination of drugs. Sorption is a combination of absorption and adsorption. Nonpolar substances are vulnerable to sorption in the plastics and rubber containers. Diazepam leaves the solution when packed in a plastic container. Similarly, the rubber stoppers used in injections cause the separation of antimicrobial preservatives from the formulation. Table 5.1 further summarizes the effect of physical instability in several dosage forms.

5.6 Stability of Drugs in Pharmaceutical Products

The stability of drugs varies in different dosage forms. The tablets of glyceryl trinitrate exhibit a phenomenon of sorption when packed in plastic containers. To keep the tablets stable throughout the shelf-life, they are preferably packed in glass bottles and aluminum-lined caps. Nifedipine in an amorphous form experiences crystal growth on exposure to humid conditions. This alteration from an amorphous form to crystal form results in modified dissolution and solubility. The crystal growth can be hindered by the addition of 2-hydroxypropyl-beta-cyclodextrin (HP-beta-CD). A similar crystallization pattern is shown by oxyphenbutazone and haloperidol acetate. Moreover, the tablets of ethenzamide, aspirin, and caffeine anhydride undergo whisker crystallization [1].

Paclitaxel, a cytotoxic drug, is stored in a glass or polyethylene infusion containers as it accelerates the diethylhexyl phthalate (DEHP) release in case of plastic containers. DEHP migrates from the plastic container into the formulation, resulting in loss of water, and hence a concentrated form of the drug is obtained upon storage. Even the glass containers may release hydroxyl ions to the dosage forms, but treated glass containers are available to minimize this issue [2].

5.7 Stability of Excipients Used in Pharmaceutical Products

The excipients are inactive ingredients present in the formulation to enhance or preserve the stability, to increase bioavailability, to improve patient acceptance, or to intensify the safety and efficacy of the dosage form. Despite the fact that excipients are chemical inert, they however also induce instability in a pharmaceutical product [14]. Excipients undergo chemical, microbial, and physical instability. The physical instability widely includes precipitation, crystallization, agglomeration, phase transformation, polymorphism, and solubility changes. Due to these instability issues, the benefit of selection or adding an excipient may not be achieved during storage [15]. The excipients fall into basic categories of diluents, binders, disintegrants, lubricants, glidants, solvents, and coating agents.

Table 5.1 Physical stability of pharmaceutical products. (Adapted from [2])

Formulation type	Physical instability	Effect on dosage form
All liquid products	Sorption of drug to container or closure	Loss of drug.
	Extraction of materials into liquid from container or closure	Possible toxicity of extractives change of pH of solution poor appearance
	Shedding of particles from glass containers	
	Evaporation of chloroform (used as antimicrobial preservative)	Potential harm to patient with injection products
		Microbial contamination
Solutions	Precipitation of drug or degradation products	Poor appearance
		Loss of efficacy
Suspensions	Caking of sediment	Inaccurate dose of drug taken by user
	Particle growth	Poor appearance
		Grittiness
Emulsions and creams	Creaming and cracking	Poor appearance
	Reduction in viscosity	Non-homogeneous product
		Increased risk of creaming and cracking
		Poor application characteristics for topical products
Ointments	Separation of liquid onto surface (bleeding)	Poor appearance
Solid dosage forms	Polymorphic change	Reduced drug dissolution rate
		May affect drug dissolution
	Change in disintegration time of dosage form	May cause change in disintegration time of tablets
		Poor appearance
	Change in crushing strength	Loss of protection of enteric coated products from gastric acid
	Cracking of coated tablets	Loss of drug
	Evaporation of glyceryl trinitrate (nitroglycerin)	
Transdermal patches	Change in drug release rate	Changed therapeutic effect
	Change in patch adhesive characteristics	Patch may not remain adhered to the skin
Inhalation and nasal aerosols	Change in particle size distribution of emitted dose	Reduced therapeutic effect

Diluents are added in the solid dosage form to increase the bulk of formulation. Microcrystalline cellulose (MCC) and lactose are the most commonly used diluents tablets. MCC is a hygroscopic compound that transforms from glass to a rubbery form at a moisture level $< 5\%$. Similarly, lactose (β-D-galactopyranosyl-(1–4)-D-glucopyranose) is unstable at higher humidity. With the increase in moisture, the

lactose crystallizes resulting in the caking of the formulation. On account of being incompatible, lactose cannot be used for aceclofenac [16], ketoprofen, and lisinopril [17]. Sorbitol accelerates the degradation of penicillins in solutions [18]. Furthermore, mannitol causes capping of the tablets upon compression [19].

Binders are added in solid dosage form to improve the flow properties of the granules. Starch is extensively used as a binder in tablets and capsules. However, certain drugs adsorb on the surface of starch resulting in poor bioavailability. For example, almost 27.6% of ketotifen adsorb on starch surface resulting in insufficiency of drug for absorption [20]. Acacia gum and tragacanth gum are also used as binders, thickeners, and suspending agents in pharmaceutical products.

Disintegrants and superdisintegrants are incorporated in tablets that require dissolution in the mouth. Carboxymethylcellulose sodium (CCS) is a commonly used superdisintegrant. However, if exposed to moisture, the CCS forms a layer on the tablet causing a delay in drug release. Similar behavior is observed with sodium salt of carboxymethyl starch on exposure to humid conditions [21].

Magnesium stearate and talc are used as lubricants in various tablets and capsules. The impurities present in them cause instability of certain drugs. Magnesium stearate accelerates the hydrolysis of acetylsalicylic acid. Similarly, it causes deterioration of quinapril on exposure to moisture [22]. Talc imparts alteration in the color of the formulation due to the presence of aluminum and iron impurities [14].

Solvents act as a vehicle for the dissolution and dissemination of drugs. Ethanol, isopropanol, acetone, and chloroform are widely used solvents in pharmaceutical manufacturing. Ethanol possesses compatibility problems with aluminum containers, whereas chloroform decomposes in the presence of air and light [18]. In the manufacturing of capsule shells, gelatin is primarily used owing to good stability properties. Gelatin must be stored properly to avoid the formation of pellicles and fluctuations in the disintegration rate [23].

Consequently, choosing an excipient requires extensive comprehension of physical and chemical debasement pathways. Information regarding the chemical nature of excipients and its effect on drug stability is essential before designing a pharmaceutical product.

5.8 Kinetic Parameters of Physical Degradation

The mechanisms of physical degradation are not studied extensively because of their complexity in nature. Therefore, the physical degradation cannot be anticipated usually on the basis of kinetic parameters. However, the transitions of polymorphs can be explained using a kinetic parameter.

Hancock-Sharp equation describes the kinetics of polymorphic transitions:

$$-\ln[\ln(1-\alpha) = \ln B + m \ln t]$$

where B is a constant and α is the fraction of drug in the product state over the fraction in the starting state [1].

5.9 Methods for Detecting Physical Degradation

The analysis and determination of drugs and degradants is a critical step in establishing stability-indicating method. Several chromatographic techniques and high-performance liquid chromatography are employed for detecting the degradation of pharmaceuticals. For complex dosage forms, differential scanning calorimetry (DSC), differential thermal analysis (DTA), and differential thermogravimetry (DTG) can be utilized to highlight the calorimetric and weight changes in the dosage form due to degradation [24–27].

Several other stability testing methods are also adopted during the manufacturing process to check the stability of pharmaceuticals. On the basis of objectives, the stability testing methods are categorized into four major classes: real-time testing, accelerated stability testing, retained sample stability testing, and cyclic temperature stress testing. Real-time testing is generally adopted to check product stability under optimum storage conditions over longer duration, whereas accelerated stability testing are performed at accelerated temperature, moisture, light, pH, and packaging to determine conditions that escalate the degradation process. Retained sample stability testing is commonly practiced before marketing a product. It requires retaining samples from a batch every year and stability is checked over specific time intervals. However, the cyclic temperature stress studies are rarely performed. These tests are designed such that a product undergoes various temperature stresses in cyclic manner. It represents different conditions that a product will encounter during 24 h of storage [28].

5.10 Conclusion

The pharmaceutical products are susceptible to physical, chemical, and microbial degradation throughout their shelf-life. However, the degradation can be minimized by several methods. The most crucial step for maintaining the desired stability is careful manufacturing. Suitable selection of excipients primarily solvents, stabilizing agents, antioxidants, or preservatives can prove effective in obtaining the desirable stability. Furthermore, appropriate packing material may also prevent degradation through moisture and light. The packaging should be inert, and no interaction occurs between packaging material and the pharmaceutical product. Prior to marketing, a product must undergo stress, short-term, and long-term stability testing to ensure its stability. In conclusion, the stability of a pharmaceutical product should be made certain for appropriate quality and efficacy during shelf-life.

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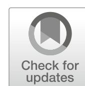

Role of Microbial Degradation on Drug Stability

6

Yusra Habib Khan, Maria Rasheed, Nasser Hadal Alotaibi, Syed Nasir Abbas Bukhari, and Tauqeer Hussain Mallhi

Abstract

The stability consideration of active pharmaceuticals and excipients is essential in the formulation of drug dosage forms. This is because the products do not remain in physical, chemical, microbiological, and therapeutic specifications due to loss of efficacy through degradation. The product becomes harmful for the patients due to degradation by microorganisms. Thus it is dependent upon the type of microbe or level of toxicity it may produce. If parenteral or ophthalmic formulations are contaminated, it may cause serious harm, but contamination in other nonsterile products is not damaging. Personnel, water used for manufacture, the processing equipment and raw materials are main sources of microflora in pharmaceuticals. Clean room staff carries dust with them into the manufacturing zone; therefore, the distribution of rod bacteria increases to grade B and D from A. Contamination with microorganism is more common in untreated raw materials during the process of manufacturing. The most common reason of contamination is high microbial and fungal count in those pharmaceutical products that contained naturally derived raw materials. Visual appearance of pharmaceuticals is also affected by microbial contamination. Therefore, use of preservative is the most commonly used way to combat microbial contamination.

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_6

Keywords

Stability · Degradation · Microorganism · Contamination

6.1 Introduction

The extent to which drug substance or product remains within its specifications (the same properties and characteristics that it possessed at the time of its manufacture) throughout period of storage and use is termed as “Drug stability”. This type of stability is generally divided into physical, therapeutic, chemical, microbiological, and toxicological. Decomposition occurs faster in pure drugs when formulated into medicines due to presence of moisture content [1].

6.2 Factors Affecting Drug Stability

Factors affecting drug stability are the following:

6.2.1 Light (Photolysis)

Various sources of light, e.g., sunlight, metal halide lamps, fluorescent light, or other indoor lightening sources, induced reactions that results in decomposition. Packing of drugs in aluminum foil wrappers, amber-colored bottles, and cardboard prevent photolysis and hence drug degradation.

6.2.2 Temperature

Oxidation, reduction, and hydrolysis reactions hasten at high temperature which causes degradation of drugs.

6.2.3 Moisture

Hydrolytic reaction is accelerated due to increase in moisture content. Packaging with glass and plastic materials prevents the exposure of drug products to humid condition [2].

6.3 Containers and Closures

6.3.1 Glass

Glass is resistant to chemical and physical change and is the most commonly used material but possesses some limitations. Decomposition enhances due to transmission of light through glass. Ions may precipitate insoluble crystals from glass.

6.3.2 Plastic

Migration of drug through plastic into environment is a major problem with plastic material. It may also result in adsorption of active ingredient or transfer of environmental moisture, oxygen, and other elements into pharmaceutical products.

6.3.3 Metals

Emulsion, ointment, pastes, and creams are kept in containers made up of aluminum tubes and alloys. Drug product undergoes corrosion and precipitation due to exposure to these metals.

6.3.4 Rubber

Leaching of containers and extraction of drug ingredients are major problems with use of rubber material [1].

6.4 Types of Drug Degradation

6.4.1 Physical Degradation

Change in physical nature of drug including appearance, particle size, brittleness, hardness, and an organoleptic property is called physical degradation. Factors affecting physical degradation are as follows:

6.4.2 Polymorphic Changes

In polymorphic changes, crystal forms are changed. The solubility and melting point of different polymorphs of the same drug may exhibit some difference. Crystal growth occurs in aqueous suspension along with change in solubility due to loss of stable crystal form, e.g., cocoa butter and chloramphenicol palmitate.

6.4.3 Loss of Volatile Components

Loss of alcohol, menthol, volatile oil and ether through vaporization at ambient temperature may also result in degradation of pharmaceuticals.

6.4.4 Loss of Water

Evaporation of water from liquid preparation results in crystallization due to change in concentration of drug. Evaporation of water from oil in water cream may cause rubbery surface appearance and hence cracking of emulsion.

6.4.5 Examples

Saturated solution: Saturated solution becomes supersaturated because loss of water results in formation of crystals.
Creams: Loss of water from oil/water cream results in dryness.
Emulsion: Separation of two phases due to loss of water.

6.4.6 Color Changes

Two types of color changes are the following:

- Development of color
- Loss of color

Exposure to light may cause development of color, while pH changes result in loss of color, e.g., phenolphthalein is colorless in acidic solution and pink in basic solution.

6.4.7 Absorption of Water

Physical degradation occurs in hygroscopic drugs due to absorption of water from external atmosphere, e.g., deterioration of powders and effervescent tablets.

6.4.8 Chemical Degradation

Change in chemical nature of the drug is referred to as chemical degradation.

6.4.9 Hydrolysis

Lactones, esters, and amides containing drugs may be susceptible to hydrolytic degradation, e.g., degradation of aspirin into acetic acid and salicylic acid.

6.4.10 Oxidation

Oxidation is defined as removal of an electropositive atom, radical, and electron or addition of an atom, radical, and electron. Oxidation has two types:

- Auto-oxidation: Oxidation in which oxygen present in air is involved
- Photo-oxidation: Removal of electron without presence of oxygen

6.4.11 Decarboxylation

Elimination of CO_2 from compound is termed as decarboxylation, e.g., decarboxylation of drug containing carboxylic acid group.

6.4.12 Isomerization

Isomerization is a process of transformation of inactive drug from active drug with different stereochemical configuration having the same structural formula.

6.4.13 Polymerization

Formation of complex molecule through combination of two or more identical molecules.

6.4.14 Photodegradation

Chemical degradation of light-sensitive drugs or excipient molecule in the presence of sunlight or room light is termed as photodegradation, e.g., sodium nitroprusside may degrade after 4 h if not protected [3].

6.4.15 Microbiological Degradation

The damage caused by microbial contamination is dependent upon the type of microbe or level of toxicity it may produce. Microbial contamination in ophthalmic and parenteral products causes serious harm as compared to nonsterile products. It

results in general spoilage such as discoloration, production of gas, odors, and breakdown of emulsions. Degradation into therapeutically inactive products involves drugs, e.g., paracetamol, salicylate, hydrocortisone, atropine, and chloramphenicol. The ready source of nutrition to microbes is preservatives especially aromatic in structure. Pyrogens released by Gram-negative bacteria represent the hazardous product and may cause fever if administered inadvertently to patient [4].

6.4.16 Sources of Microbiological Contamination

Microbial count affects the quality of pharmaceutical and cosmetic products during manufacturing process. The sources of microflora in pharmaceutical products include (1) the personnel, (2) water used in manufacturing, (3) the processing equipment, and (4) raw material [5]. Table 6.1 describes few sources of microbial contamination along with microorganisms.

6.5 Microorganisms in Manufacturing Sites

6.5.1 Microorganisms in Clean Room

Human skin flora (Gram-positive cocci) are responsible for contamination of grade A and grade B clean rooms [6]. Despite protective equipment such as head cover, gowns, and gloves, *Micrococcus* and *Staphylococcus* (82%) are predominant in working zone. Clean room staff carries dust with them into the manufacturing zone; therefore, the distribution of rod bacteria increases to grade B and D from A. The presence of a water source within rooms such as water outlet sinks and steam machines is a source of Gram-negative genera in grade C and grade D clean rooms [7].

6.5.2 Microorganisms in Raw Materials

Contamination with microorganism is more common in untreated raw materials during the process of manufacturing. These microorganisms may cause contamination of final products if not manufactured under GMP regulations [8]. The highly

Table 6.1 Contamination sources

Sources	Microorganisms
Personnel	Staphylococci, Streptococci
Water	Gram-negative bacteria, *Pseudomonas*, *Xanthomonas*
Air	*Penicillium*, *Aspergillus*, *Bacillus* species, yeast
Raw materials	Micrococci
Animal products	Salmonella, Coliforms

toxic material such as aflatoxin B is produced by *Aspergillus* sp. detected in raw materials [9]. Improper handling of raw materials obtained from plants affects the quality of final product due to presence of microorganism [10].

6.5.3 Microorganisms in Pharmaceutical Products

6.5.3.1 Pharmacy Stores

Pharmaceutical products after reaching market should be routinely monitored in order to assess the microbial count. The most common reason of contamination is high microbial and fungal count in those pharmaceutical products that contained naturally derived raw materials [11].

6.5.3.2 Hospitals

Final product is exposed to microflora inside hospitals due to compounding products from commercialized large-batch products. Microbial count is related to human activity. In intensive care unit, microbial count is lowest due to restricted human activity [12–15]. On the contrary, microbial count is much higher in other areas, which have frequent introduction of outdoor materials [12–17]. The predominant bacterial genera include *Bacillus*, *Staphylococcus*, *Corynebacterium*, and *Micrococcus* [12–14]. The predominant fungal species include *Aspergillus*, *Alternaria*, *Penicillium*, and *Cladosporium* [12–17].

6.6 Role of Microbiological Contamination

6.6.1 Change of Activity

The formation of toxic products results in change in activity of active ingredients such as loss of activity or deleterious effects. These toxic products are a result of microbial attack of pharmaceuticals [4].

6.6.2 Acetylsalicylic Acid

Acetylsalicylic acid is used as an analgesic and indicated for treatment of cardiovascular diseases [18]. *Acinetobacter lwoffii* bacteria cause degradation of aspirin by hydrolysis. Soluble intracellular esterase of *Acinetobacter lwoffii* is responsible for microbial degradation of aspirin [19].

6.6.3 Caffeine

Caffeine is used for treatment of migraine headaches. It is used in combination with NSAID as an analgesic and as central nervous system stimulant in beverages

[20]. Caffeine undergoes fungal degradation leading to formation of theophylline by deriving source of nitrogen which has higher toxicity [21].

6.6.4 Hydrocortisone

Hydrocortisone is used as an anti-inflammatory and antipruritic for the treatment of common dermatomes [22]. The oxidative enzymes produced by *Clostridium* species cause degradation of hydrocortisone resulting in formation of androst-4-ene-IIβ-o1-3:17-dione [23].

6.6.5 Progesterone

Conversion of progesterone to testosterone through oxidation of 20-ketones to 17-β acetates is caused by microbial attack of *Penicillium* and *Aspergillus* species [24].

6.6.6 Atropine

Pseudomonas metabolizes atropine due to cleavage of ester bond resulting in formation of tropic acid and tropine. Pseudomonas metabolizes atropine and results in formation of tropic acid and tropine due to cleavage of ester bond [25].

6.6.7 Visible Effect

One of the important organoleptic properties of pharmaceutical products is appearance. Visual appearance of pharmaceuticals is affected by microbial contamination. These changes compromise patient health and safety due to deviation from targeted pharmacokinetics [26].

6.6.8 Visible Growth

The manifestation of microbial contamination is the formation of colored colony, pellicle colony, and appearance of clear sediment. Bacteria, yeast, molds, and algae make clear solution turbid [4].

6.6.9 Loss of Consistency

Loss of required consistency is caused by microbial attack and results in therapeutic failure. Thick consistent gels transform into slippery liquids by *Penicillium*, *Aspergillus*, and *Fusarium* species [26].

6.6.10 Separation of Phases

Pseudomonas not only produce foul odor due to deterioration but also cause breaking up of emulsions. *Pseudomonas* breaks hydrocarbons and fats and is capable of liquefying gelatin. *Trichoderma viride*, *A. niger*, *Aspergillus flavus*, and *Pseudomonas aeruginosa* also cause phase separation of oil-water olive emulsions [27].

6.7 Approaches to Counter Microbial Instability

6.7.1 Use of Silver Nanoparticles

The antimicrobial activity of silver nanoparticles was evaluated by using a mixture of bacterial suspension (containing *P. aeruginosa*, *S. aureus*, and *E. coli*). The test was carried out by using 20 g of silver nanoparticles. These particles were inoculated with these silver nanoparticles after aseptically transferring to three sterilized vials. Incubation of vials at 30 °C for bacterial suspension and at 25 °C for fungal suspension was required for measurement of readings. The desirable number of microbial colonies was observed in test vials that contained silver nanoparticles [28].

6.7.2 Increase in Paraben Efficacy

Pilocarpine eye drop containing paraben when inoculated with *P. aeruginosa* and *C. albicans* showed that minimum inhibitory concentration met acceptable limit according to European Pharmacopoeia [28].

6.7.3 Use of Zinc Oxide

Zinc oxide possesses low toxicity, easy clearance, and high antimicrobial activity. Therefore, it can be used as preservative in pharmaceuticals [29, 30].

6.8 Conclusion

Biological contaminants to pharmaceuticals and cosmetic products are microorganisms. There is an acceptable contaminated level depending on the type of products. Mild (changes in appearance), to moderate (loss of consistency), to severe changes (change in activity and toxicity in degraded products) are observed in pharmaceuticals products due to microbial contamination. The preventive methods depend upon the type of microorganisms (Gram positive could be prevented by minimizing human presence, and fungi could be controlled by reducing moisture) as well as potential contaminant sources. Use of preservative is the most commonly

used way to combat microbial contamination. However, hydrolysis of preservatives by microorganism causes development of resistance in antibiotics. Hence, researchers are prompted to adopt other strategies for reducing microbiological contamination in pharmaceuticals.

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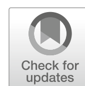

7 Role of Decomposition on Drug Stability

Yusra Habib Khan, Abrar Ahmad, Muhammad Hammad Butt, Shahzadi Misbah, Muhammad Shahid Iqbal, and Tauqeer Hussain Mallhi

Abstract

Degradation and decomposition are interchangeably used in pharmaceutical sciences and are defined as the physical or chemical processes that lead the product to decline in its intended quality characteristics, which are claimed by the manufacturer. Both physical and chemical degradation reactions affect the stability of drugs. Physical degradation includes loss of water and volatile compounds, polymorphic changes, crystal growth, change in colour and water absorption. On the other hand, chemical degradation processes include hydrolysis, oxidation, decarboxylation, elimination, isomerization, dimerization, epimerization, photodegradation and dehydration. Various pharmaceutical dosage forms demonstrate different mechanisms of drug decomposition or degradation.

Keywords

Drug stability · Shelf life · Types of drug stability · Factors affecting on drug stability

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_7

7.1 Introduction

Drug stability may be defined as the ability of a drug product or formulation to retain its properties such as physicochemical, microbial and biological (therapeutic and toxicological) activity within a specified standard range throughout its shelf life when stored and used according to specifications. In other words, the pharmaceutical product should remain in the same specified standard limits of properties and condition from its production and packaging according to quality-control specifications till its expiry date [1, 2]. The expiry date of a drug product may be defined as the date beyond which the product is not guaranteed to have the same properties and characteristics as the labelled information, rendering it ineffective and in some cases even toxic due to degradation into unintended toxic chemicals. Products past their expiry date are therefore not fit for use and consumption.

It is also very important to mention here that the product, if not stored according to the specified instructions on the label by the manufacturer, may become unfit for use even well before the expiry date. An example of such products is ophthalmic drops which when opened become unfit for use after a month, which can be well before the expiry date [2].

The shelf life of a drug product may be defined as the time duration in which the product is guaranteed to maintain its efficacy and safety profile, given that the product is stored and used according to the labelled instructions by the manufacturer [3]. Degradation is defined as the physical or chemical processes that lead the product to decline in its intended quality characteristics, which are claimed by the manufacturer. Pharmaceutical degradation may be defined as the processes that might be involved in causing deviations in physicochemical, microbial or biological properties and characteristics beyond the specification ranges which are claimed by the manufacturer on the label.

7.2 Types of Stability

Bearing in mind the wide variety of pharmaceutical formulations and products in various physical forms, according to USP (United States Pharmacopeia), stability is commonly categorized into the following [4].

7.2.1 Physical Stability

Physical stability involves the retaining ability of a pharmaceutical product of its physical properties and to resist changes through physical processes. Physical properties vary with the nature or type of formulation in a product and may include physical appearance, uniformity, palatability, dissolution, etc. [5].

7.2.2 Chemical Stability

Chemical stability is the ability of the pharmaceutical product to retain its chemical properties within specified standard ranges or limits given in the monograph or product label. The chemical properties involved include the potency, concentration, integrity, etc. of all the chemical ingredients specified in the composition [6].

7.2.3 Microbiological Stability

Microbiological stability of pharmaceutical products includes their sterility or resistance within a specified range or limit against potential microbial growth, both due to the effect of preservatives or other excipients and packaging or container design. For products containing an active antimicrobial agent, microbiological stability includes the retaining of efficacy of that agent within a specified limit [7].

7.2.4 Therapeutic Stability

Therapeutic stability includes the retention of the therapeutic effects which are essential for the efficacy of the pharmaceutical product. The pharmaceutical product is supposed to have therapeutic effect as specified in the official monograph or by the manufacturer.

7.2.5 Toxicological Stability

Toxicological stability includes the absence of any significant toxic material resultant from degradation or due to any other reason the increase in the toxic effects of the drug.

7.3 Factors Affecting Drug Stability

Stability of pharmaceutical products can be affected by various factors which impart some sort of physical or chemical change within the product formulation when exposed to specific conditions. Such factors include temperature, pH, moisture, light, concentration, dosage form, drug compatibility, oxidizing or reducing agents, etc.

7.3.1 Temperature

Temperature is one of the most important factors that affect the stability of drugs. Extreme temperature in some regions affects the product features. When the storage

temperature exceeds above or below the specified temperature range, physical or chemical degradation might accelerate, rendering the drug product ineffective or unsafe. Often, products are advised to be stored at room temperature of around 25 Celsius or temperature range of 2–8 Celsius in a refrigerator. In some cases, specific products are even frozen well below 0 Celsius [8–10].

7.3.2 pH

The pH of the medium of the pharmaceutical products affects the ionization of many compounds, and they, when in ionized form, are more prone to undergo chemical reactions resulting in degradation and instability. Some components precipitate when changed to unionized form. Many drugs are stable between pH 4 and 8 [11].

7.3.3 Moisture

Moisture and humidity can impart changes to certain pharmaceutical products, causing physical, chemical or microbiological degradation. A common example may be effervescent dosage forms, which when exposed to moisture result in deterioration [12].

7.3.4 Light

Electromagnetic radiations on the lower wavelength spectrum have higher energy, and when products containing certain susceptible chemicals are exposed to such radiations as UV from sunlight, they may undergo degradation by absorbing energy from photons, which initiates a decomposition reaction [13].

7.3.5 Concentration

Concentration of active ingredient in a pharmaceutical product also affects the stability. Commonly, the degradation rate for a solution of an ingredient is the same regardless of the concentration. Therefore, concentrated stock solutions tend to be more stable and diluted solutions degrade rapidly as higher ratio of active agent degrades [13].

7.3.6 Pharmaceutical Dosage Form

Some dosage forms are more susceptible to degradation and consequently decreased stability. Solid dosage forms usually have higher stability because of relatively decreased water content [13].

7.3.7 Drug Incompatibility

Degradation can also occur as a consequence of interactions between two or more ingredients within the dosage form or between ingredients and the enclosing primary packaging or container [13].

7.3.8 Oxygen

Among the ingredients of a formulation in a dosage form, some might be more prone to oxidation when exposed to air containing oxygen. To avoid such degradation and instability, containers are mostly airtight and in some cases, such as in single-use ampoules, nitrogen gas, which is an inert gas, is used to fill the container to remove oxygen [14].

7.4 Types of Degradation Reactions

Degradation processes can be divided into two on the basis of their nature.

7.4.1 Physical Degradation Reactions

Often, due to mostly the environmental or other factors, the product undergoes physical degradation. The following are some of the examples of such degradations.

7.4.1.1 Loss of Water

When the product is exposed to open air or the container is not airtight, some formulations might lose their water content to evaporation. This water loss results in consequent increase in the concentration of the drug or other excipients which causes crystallization. Oil in water emulsions formulations might undergo cracking or other instabilities.

7.4.1.2 Loss of Volatile Components

Similarly, volatile substances such as organic solvents might evaporate, hence, making the dosage form unstable in some way. Examples of such dosage forms include spirits, elixirs and aromatic waters. Solid dosage forms might also contain volatile substances, such as in nitroglycerine tablet.

7.4.1.3 Polymorphic Changes

Some drugs might have more than one crystalline form, and may interchange between them depending upon certain factors. Different crystalline forms have varying physical properties and this change in physical properties upon interchanging of forms affect the stability, efficacy or safety of the formulation. A common example of such a drug is chloramphenicol.

7.4.1.4 Crystal Growth

Upon loss of water or volatile substances or change in temperature, some active agents in formulations crystallize and make the formulation unstable. For example, stearic acid in carbamazepine tablets, which crystallizes when exposed to high temperature.

7.4.1.5 Colour Changes

Sometimes due to several environmental factors, some formulations might change in colour (lose or develop a colour). Usually, loss of colour is caused by changing pH while exposure to light might cause some colour development. For example, phenolphthalein becomes colourless in an acidic environment and pinkish in an alkaline environment.

7.4.1.6 Absorption of Water

Some dosage forms containing components that are hygroscopic, having the tendency to absorb water, degrade when exposed to moisture or humid environment. Powders in particular are vulnerable to this degradation type. Suppositories containing hydrophilic components, such as gelatine, polyethylene glycol, glycerine, etc., undergo this type of degradation, and their consistency becomes jelly-like [13, 15].

7.4.2 Chemical Degradation Reactions

Pharmaceutical products contain components that include active agents and excipients which are essentially chemicals of varying molecular structures possessing a variety of functional groups. Because of their nature, these components may undergo some type of chemical reactions when exposed to certain environments, media or other factors. Examples of chemical degradations are hydrolysis, oxidation, decarboxylation, elimination, isomerization, epimerization, photodegradation, dehydration, etc.

7.4.2.1 Hydrolysis

One of the most common chemical degradation processes in pharmaceutical products, especially those containing ester, amide, imide, carbamate, lactone, nitrile and carbohydrate groups, is hydrolysis. It involves degradation by water. Many pharmaceutical products may undergo acid or alkaline hydrolysis when exposed to aqueous environment or moisture. Examples of such drug products include paracetamol, sulfacetamide, indomethacin, procaine, digoxin, riboflavin, lincomycin, chloramphenicol, penicillin, cephalosporins and benzodiazepines [13].

Some functional groups that are subject to hydrolysis are described in Table 7.1.

7.4.2.2 Oxidation

Oxidation is another common way of chemical degradation in pharmaceutical products when exposed to oxygen or other oxidizing agents. The oxidation process

Table 7.1 Functional groups and their examples

Functional group	Example
Esters	Aspirin, alkaloids – nitroglycerine Dexamethasone sodium phosphate
Lactones	Pilocarpine – spironolactone Amides – chloramphenicol
Lactams	Penicillin – cephalosporin
Imides	Glutethimide

can occur at any stage, from manufacturing till consumption, when exposed to air or an environment containing oxidizing agents. The oxidation reaction rate may also be affected by the pH of the medium as a consequence of ionization and change in the redox potential of the chemicals that are involved. Examples of drugs that may undergo oxidation include ascorbic acid, vitamin A, glucose, morphine, hydrocortisone, methyldopa, aldehydes, phenols, unsaturated compounds, thiols, phenothiazines and polyenes [13].

7.4.2.3 Decarboxylation

Elimination of carbon dioxide from a chemical compound is called decarboxylation. Pharmaceutical products that contain chemical components possessing carboxylic acid functional group are susceptible to this form of chemical degradation. An example of such a product would be a pharmaceutical product of 4-aminosalicylic acid which when exposed to an aqueous medium, undergo decarboxylation reaction and form 3-aminophenol. The pH of the media also affects decarboxylation as they are more ionized in alkaline media [16].

7.4.2.4 Elimination

Removal of substituents from a compound is called elimination reaction. They may occur in a single or double step. The single-step elimination involves the E2 bimolecular reaction and the double-step elimination involves the E1 unimolecular reaction. Pharmaceutical products containing organic compounds may sometimes undergo this type of chemical degradation. Trimelamol, which is a synthetic antitumor drug, is an example of a pharmaceutical product that may undergo elimination reaction. It undergoes elimination of formaldehyde to form trimethyl melamine [17].

7.4.2.5 Isomerization

The process by which molecules of a compound transform to another form by rearranging without change in the molecular formula is known as isomerization. Due to isomerization, when a drug compound changes to another form, it might lose its efficacy. Cephalosporins are an example of such drug compounds.

7.4.2.6 Dimerization

When two initially separate molecular units join to form a single molecular unit, it is called dimerization. Nalidixic acid is an example of such drug compounds. It undergoes dimerization when exposed to high temperature and forms a dimer of two decarboxylated units of nalidixic acid.

7.4.2.7 Epimerization

Changing of just one of the chiral centres of a molecule to form another similar molecule called epimer with a different orientation of the atoms on that carbon is known as epimerization. Ergotamine is an example of such drug compounds. It undergoes epimerization at various chiral centres in the molecular structure under certain conditions.

7.4.2.8 Photodegradation

Pharmaceutical products may also contain components that are sensitive to light from the sun or an artificial source, as light especially on the lower spectrum of the wavelengths such as UV has higher energy, and when certain compounds absorb this energy, a chemical reaction might initiate which can lead to degradation. Photodegradation mostly involves oxidation mechanism; however, others like polymerization or ring opening may also occur. Photodegradation may occur at any stage, from the manufacturing till consumption, when exposed to light. For this reason, light-sensitive pharmaceutical products have amber-coloured or some other coloured containers if transparent. Nifedipine, nicardipine, nitroprusside, chlorthalidone, acetazolamide, retinol, riboflavin, furosemide and phenothiazines are examples of compounds that are light-sensitive [18, 19].

7.4.2.9 Dehydration

The chemical process in which loss of water molecule from the parent molecule occurs is known as dehydration. For example, batanopride hydrochloride, an antiemetic drug compound, undergoes dehydration to form 2,3-dimethylbenzofuran. Prostaglandin E2 and tetracycline are other such examples [20].

7.5 Compatibility of Drug with Packaging Material

To protect the pharmaceutical products from degradation by the effects of environmental factors, products are enclosed in containers or other forms of primary packaging. However, the material of the immediate enclosing packaging should also be taken into consideration as it may sometimes be incompatible with components of the formulation and lead to degradation. Common types of packaging materials may be glass, plastic, rubber (natural and synthetic) or metal [1, 13].

7.5.1 Glass

One of the most compatible of all the materials is glass because of its resistance to chemical and physical changes. However, it too has some limitations:

- It has some alkalinity which might affect the pH of the pharmaceutical product.
- Ionic radicals that are present in the drug may precipitate insoluble crystals by reacting with components from the glass.
- Glass material is often transparent and allows high energy wavelengths of light to pass through which can result in photodegradation.

These limitations can be overcome through the following ways:

- Borosilicate glass which has lesser amounts of reactive alkali ions can be used to prevent the problems of alkalinity.
- Treated glass or buffers can be used to prevent the reaction of ionic radicals with glass material.
- To prevent photodegradation, amber-coloured glass can be used as it only allows light above 470 nm wavelength which do not have high energy.

7.5.2 Plastic

Plastics are of many varieties made up of various polymers having different densities, molecular weights and physicochemical characteristics. They can cause some problems such as:

- Leaching of materials from the plastic container into the pharmaceutical product medium.
- Drug might migrate to the environment through the plastic material.
- Components of air or environment might transfer into the pharmaceutical product through the plastic.
- Components of the pharmaceutical formulation might get adsorbed or absorbed by the plastic material.

To prevent the above-mentioned problems, compatibility of components of formulation with the container material must be tested and plastic material could be treated chemically.

7.5.3 Rubber

Rubber is used mostly as stopper for vials. Rubber has similar problems as those of the plastic material. It too can leach ingredients to the pharmaceutical product medium or extract components from the pharmaceutical formulation. These

problems can be avoided by using neoprene, butyl or natural rubber, coated with certain epoxy, Teflon or varnish. Surface blooms and leaching can be reduced by the pretreatment of rubber stoppers and closures with water and steam.

7.5.4 Metal

Aluminium, other metals and their alloys are often used in the packaging material for pharmaceutical products such as emulsions, ointments, creams and pastes. Such packaging material may undergo corrosion and consequent precipitation in the pharmaceutical formulation. Such corrosion and precipitation can be prevented by coating the metal with polymers or epoxy.

7.6 Degradation Mechanisms in Pharmaceutical Dosages Forms

7.6.1 Oral Dosage Form

7.6.1.1 Problem

- Precipitation
- Loss of flavour
- Change in taste
- Discoloration
- Loss of dye

7.6.1.2 Prevention

By selecting the appropriate packaging material and proper excipients, the abovementioned problems can be resolved.

7.6.2 Parenteral

7.6.2.1 Problem

- Discoloration
- Presence of precipitate
- Presence of whiskers

7.6.2.2 Prevention

The use of the following materials increases the stability of parenteral drugs:

- Chelating agent
- Antioxidants
- Suitable material
- Co-solvents

7.6.3 Emulsions

7.6.3.1 Problem

- Cracking
- Creaming

7.6.3.2 Prevention

By selecting right emulsifying agents, the abovementioned problems of emulsion can be resolved [13].

7.7 Role of Pharmacist

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines say that pharmacists should be playing their significant role in ensuring the quality, efficacy and safety of the pharmaceutical products that are dispensed under their supervision. They should be aware of the factors and certain climatic conditions that can cause degradation in the pharmaceutical products. They should make strategies to prevent such degradations leading to instabilities. They should ensure proper storage conditions as required for the varieties of pharmaceutical products throughout their shelf life so that no harm can reach the consumer [21].

7.8 Conclusions

Drugs and pharmaceutical products are essential chemical substances that are subject to physical or chemical changes under certain storage conditions and other influencing factors as described in this chapter. Owing to their use in special conditions and consumers, i.e., diseased states where health status is not necessarily optimal and the consumer is extremely vulnerable and may suffer greatly in various ways, the understanding of mechanisms behind degradations of drugs and pharmaceutical products is crucial to determine shelf life, storage conditions and optimum packaging material selection.

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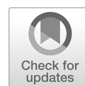

Role of Catalysis and Catalytic Agents in Drug Stability

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Sana Ghayas, Kiran Qadeer, and Zubair Anwar

Abstract

Catalysis is the phenomenon of an increase in the rate of reaction with the addition of any external chemical/biochemical substance (i.e., catalyst). These external substances (temporary intermediate) in the chemical reactions/biological processes provide an alternative pathway with lower activation energy. Catalytic agents help in the lowering of the energy of the reaction to reach the transition state with no change in the free energy from the formation of reactants to the final products. These catalytic agents help in the speeding up of chemical reaction/ biological process and also help in the production of some important pharmacologically active compounds for the treatment of many ailments. Drug discovery is the foundation of development in the healthcare system. Multiple techniques and methodologies have been adopted to achieve the balance drug molecule. However, functionally catalytic agents performed a novel mechanism in the field of drug discovery and stability. Stability continues to impact on the drug discovery and development greatly. New catalytic agents (i.e., organometallic enzymes, engineered genes, transition metal, and their salts) have been developed to accelerate the growth and development of stable compounds. The catalyst works by opening up a route between starting material and product with a lower activation barrier than the un-catalyzed process. This chapter gives detailed information to understand the catalysis, types of catalysis, the effect of catalytic agents on drug discovery, and the stability of drugs and drug substances.

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_8

Keywords

Catalysis · Types of catalysis · Drug discovery · Stability of drugs · Drug degradation

8.1 Introduction

Catalysis shows a significant role for the benefit of mankind and its role to develop a connection between science and technology with worldwide development and modernization. Different industrial development based on the catalysis process because over 90% of products have been originating depends somewhat on the catalytic process such as chemical, agriculture, polymer, petroleum, and pharmaceuticals [1]. The benefits of the catalytic process include cost minimization, time effectiveness, and reduced waste formation to make it an environmentally safe and sustainable manufacturing process. Catalysis is based on green chemistry principles [2]. Catalysis provides a helpful tool in smart synthetic design in which we obtained high value product output with simpler methods, therefore improvising the various industrial manufacturing processes. The significance of catalysis is valued by honoring Nobel Prizes in various fields of chemistry in the last 40 decades. These include the revolutionary efforts in polymers by Ziegler and Natta (1963); the remarkable work on organometallic compounds by Wilkinson and Fischer (1973); the hydrogenation and oxidation approaches of Knowles, Noyori, and Sharpless (2001); the award given to Chauvin, Grubbs, and Schrock (2005) for metathesis; the latest acknowledgment on cross-coupling reactions of Heck, Negishi, and Suzuki (2010); and many more [3].

The catalytic reaction is a cyclic process as catalyst takes part in the reaction and is available in its original state after reaction and could be used again and again in other reactions. A catalyst speeds up a chemical reaction by making an intermediate with the reactants and enables them to form a product [4]. The process of accelerating the speed of any reaction caused by the addition of a substance is termed as catalysis [5]. Catalysts speed up the chemical reactions by providing another pathway during the reaction having lower activation energy than a chemical reaction that proceeds in the absence of a catalyst [6], whereas a substance which decreases the speed of a chemical reaction is termed as an inhibitor rather than a catalyst [5].

8.1.1 Unit of Catalytic Activity

Catalytic activity (CA) is typically symbolized by “z” and calculated in mol/s, which is in SI unit termed as “Katal” (1 kat is equivalent to 1 mol/s). One katal is the amount of catalyst, i.e., one mole of catalytic agent and reactant is converted into the final product in one second. The catalyst productivity could be measured by the turnover number (TON) which is measured in one second and CA is calculated as turnover frequency (TOF) [7].

8.2 Working Principle

Catalysts typically react with more than one reactant in a chemical reaction to generate intermediates that convert into final product by recovering the catalyst at the end of reaction [8]. The classical representation of a reaction scheme is described here, where ***C*** is the catalyst, A and B are reactants, and Z is the final product (Eqs. 8.1, 8.2, 8.3, 8.4, and 8.5):

$$\mathrm{A} + C \rightarrow \mathrm{A}C \tag{8.1}$$

$$\mathrm{B} + \mathrm{A}C \rightarrow \mathrm{AB}C \tag{8.2}$$

$$\mathrm{AB}C \rightarrow \mathrm{C}Z \tag{8.3}$$

$$C\mathrm{Z} \rightarrow C + \mathrm{Z} \tag{8.4}$$

The catalyst is utilized at the beginning of a reaction (Eq. 8.1) but is recovered at the end of chemical reaction (Eq. 8.4), so it is not represented in the overall equation of the reaction:

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{Z} \tag{8.5}$$

8.3 Reaction Energetics

The reaction energetics in the catalyzed reaction is based on difference in the transition states and by lowering the activation energy. When molecular collisions are increased during the chemical reaction, the energy requirement is also increased to attain the desired transition state. Therefore, catalyst facilitates the process of chemical reaction which might be blocked or slowed down due to the kinetic barrier. So, it can increase the rate of reaction or proceed the chemical reaction at lower temperature [9]. The catalyst may also stabilize the transition state as well as cause reduction in the kinetic barrier by lowering the energy levels difference between reactants and their transition state. This reaction energetics can be explained by using the energy profile diagram (Fig. 8.1) [6].

8.4 Types of Catalysis

The two major types of catalysis are heterogeneous and homogeneous catalysis, which are described below.

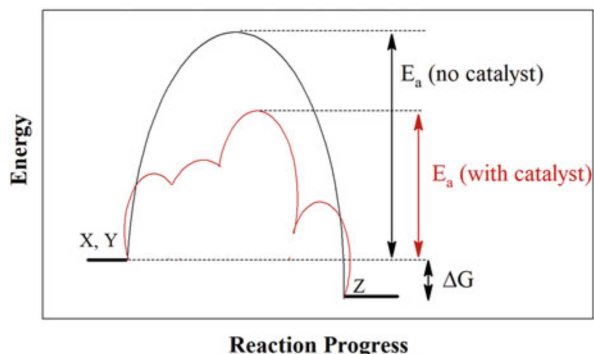


Fig. 8.1 Reaction energetics in the presence and absence of catalyst

8.4.1 Heterogeneous Catalysis

In heterogeneous catalysis, catalysts exist in a different phase than the reactants. Catalysts used in heterogeneous catalysis are solid and able to participate in a chemical reaction where substrates are in a liquid or gaseous phase. The mechanism of heterogeneous catalysis is based on surface phenomenon, i.e., adsorption [10]. This is a cycle of molecular adsorption, reaction, and desorption occurring at the catalyst surface. The surface area of heterogeneous catalyst plays a vital role on the speed of chemical reaction. The active sites present on the heterogeneous catalyst may be either a planar metal surface or a crystal edge or a complicated combination of these two. Some studies on heterogeneous catalysis for various pharmaceutical compounds include enhanced ibuprofen (IB) removal by heterogeneous-Fenton process over Cu/ZrO_2 and Fe/ZrO_2 catalysts [11]; base metal–catalyzed hydrogen isotope exchange [12]; heterogeneous copper-catalyzed C–S coupling via insertion of sulfur dioxide [13]; shape-controlled metal nanocrystals [14]; catalytic ozonation of diclofenac (DF), sulfamethoxazole (SM), and 17α-ethynylstradiol in the presence of a commercial γ-Al_2O_3 and a synthesized Co_3O_4/Al_2O_3 catalyst [15]; oxidation treatment of fluoroquinolone using TiO_2 [16]; synthesis of deuterium-labeled drugs by hydrogen–deuterium (H–D) exchange [17]; furan synthesis [18]; and palladium-derived catalysts in the synthesis of fine chemicals, pharmaceutical intermediates, and active pharmaceutical ingredients [19].

8.4.2 Homogeneous Catalysis

Homogeneous catalysts worked on the same mechanism as in heterogeneous catalysis. In this type of catalysis, the reactants are mostly in liquid phase/system. Homogeneous catalysis is frequently indistinguishable with the process of organometallic catalysis [20]. Usually, organic catalysis needs a higher amount of catalyst (per % mol of reactant) as compared to transition metal catalysis where catalysts are available in major quantities to reduce the cost of the entire process. Homogeneous catalysis is more efficient as it is highly selective in specific product formation,

enhances catalytic activity, and generates heat if required in case of exothermic reactions and the characterization of species because of the reaction is in the same solution/phase. Some examples of homogenous catalysis are perovskite catalytic ozonation of diclofenac and 17α-ethynylestradiol [21], formation of alkylaldehyde by hydroformylation with alkene and carbon monoxide, formation of methyl acetate by esterification of carboxylic acids [22], and application of homogeneous catalysis in supercritical fluids [23]. Homogenous catalysis is of different types (i.e., general acid and base catalysis, Diels-Alder reactions, thiazolium ions in cannizarro reactions, epoxidations, hydroxylations and polyester condensations). However, among them, the general acid–base catalysis is of greater implication in the discovery and stability of pharmaceutically active compounds; this is discussed below.

8.4.3 General Acid and Base Catalysis (Ester Hydrolysis)

Acids (Lewis acids) and bases act as powerful catalysts for a variety of chemical reactions, in the laboratory, industry, and processes occurring in nature. Historically, catalytic action was regarded as one of the essential characteristics of acids. However, in the nineteenth century, the parallel occurrence of catalytic action and electrical conductivity was one of the important evidences in establishing the theory of electrolytic dissociation as the basis of acid–base catalysis [24].

Acid–base catalysis was initially unknown and considered as an influence of acid or base on the catalysis. However, it is now believed that acid–base reaction is in between the catalyst and reacting substance (substrate). Nowadays, the knowledge of reaction mechanisms is sufficient to suggest detailed sequences of reactions for acid or base catalysis. In most acid–base reactions, the addition or removal of a proton does not bring about any drastic change in the structure of the molecule or its stability or reactivity. However, the addition or removal of a proton may result in the instability of substrate (due to decomposition or rearrangement) and reactivity toward some other species present in the system. In cases of rearrangement, the regeneration of the catalyst often involves the removal or addition of a proton at a site other than that at which the initial addition or removal took place. It is not necessary that the substrate in an acid- or base-catalyzed reaction should itself have marked acid–base properties, since even a very small extent of initial acid–base reaction may be enough to bring about the subsequent change [25, 26].

8.4.4 Other Types of Catalysis

Some other types of catalytic processes which are initiated with external stimuli (i.e., light, heat, etc.) or by the influence of non-catalytic agents are described below.

8.4.5 Photocatalysis

Photocatalysis is the catalysis in which the catalyst is activated by light (i.e., UV or visible light). The photoactivation of reactants results in the transformation from the ground state into excitation states through intersystem crossing (ISC). Therefore, this activation results in a series of chemical cascades that could not be possible without photoactivation of reactants. Through this phenomenon, the molecular oxygen ($^3O_2$) is converted into singlet oxygen ($^1O_2$) and widely used in photodynamic therapy (PDT) [27]. Few examples of photocatalytic reactions include removal of cytotoxic drugs from wastewater [28], antibacterial activity of metal oxide nanomaterials (NMs) [29], degradation and inactivation of tetracycline by TiO_2 [30], degradation of lincomycin (LM) in aqueous medium [31], titanium dioxide photocatalysis [32], and water purification by semiconductor [33].

8.4.6 Biocatalysis

Biocatalysis may be either homogeneous or heterogeneous. Soluble enzymes work on the principle of homogeneous catalysis and some of membrane-bound enzymes are categorized as a heterogeneous catalyst [34]. Screening of biocatalyst may be carried out by various instrumental techniques, i.e., chromatography, capillary electrophoresis, mass spectrometry, etc., through absorption or emission of light as the reaction proceeds [35]. Some monoclonal antibodies behave as a weak catalyst by lowering its activation energy in chemical reactions where the binding target is stable [36, 37]. The advancement in the designing of the biocatalyst is achieved through rational selection and mutation by recombinant DNA technology which facilitates the production of the process-compatible enzymes [38].

8.4.7 Nanocatalysis

The fabricated NMs that are used to accelerate the chemical reactions are termed as nanocatalyst and the phenomenon is known as nanocatalysis. Studies have been reported on the catalytic effect of nanocatalysts in different reactions [39]. Examples of nanocatalysis are the effect of gold nanocatalysts in the detection of mefenamic acid (MA) in pharmaceutical preparations [40] and selective transfer of hydrogen in functionalized nitroarenes using cobalt-based nanocatalysts [41].

8.4.8 Tandem Catalysis

Tandem catalysis is generally categorized under the homogeneous catalysis. In this catalysis, multiple catalysts are involved in a chemical reaction to form a product [42]. A porphyrin porous organic polymer with bicatalytic sites for highly efficient one-pot tandem catalysis [43], asymmetric coupling of ethylene and enynes to

functionalized cyclobutanes [44], direct catalytic synthesis of imines from alcohols using manganese octahedral molecular sieves [45], and homo- and heterodinuclear complexes with triazolyl-diylidene [46] are some examples of tandem catalysis.

8.4.9 Autocatalysis

Autocatalysis is the process in which the catalysts are formed during the reaction as an end product which accelerates the chemical reaction in the forward and backward direction [47, 48]. Formose reaction [48], autocatalytic degradation of obidoxime [49], and controlled autocatalytic nitration of phenol in a microreactor [50] are a few examples of autocatalysis.

8.5 Importance of Catalytic Agents in Drug Discovery and Stability

Drug discovery involves the learning of scientists about the dysfunction of biological targets (i.e., receptors, enzymes, proteins, genes, etc.) which are directly involved in the biological process. The development of a new drug produces a novel mechanism of action which is entirely different from the priorly approved drug for any ailments indicating the importance of drug discovery. Medicines with some modifications are more efficient in the domain of potency, safety, tolerability, and convenience (i.e., palatability, reduction in frequency, fixed-dose combination (FDC), reduction in the cost of therapy and hospitalization) [51].

Stability is the most important attribute of drug discovery and for potential compounds used in the treatment of diseases. The instability of the drug leads to the degradation that may cause rejection of compound in a later stage of development and would cause some major problems in the biological system. The importance of stability studies in drug discovery is to recognize responsive chemotype, important lead series, to detect precise structure–activity relationship (SAR) due to degradation, to improve stability by structural modification, and to elucidate the mechanism of action [52].

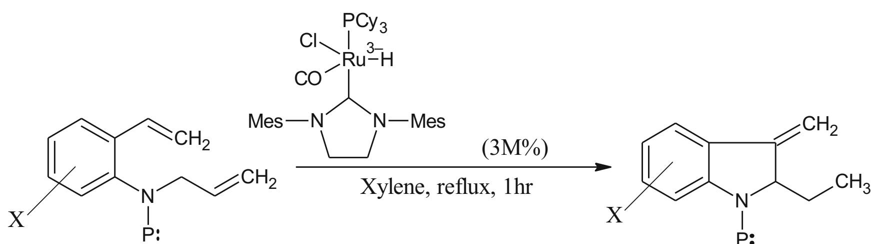
8.5.1 Challenges in Drug Discovery

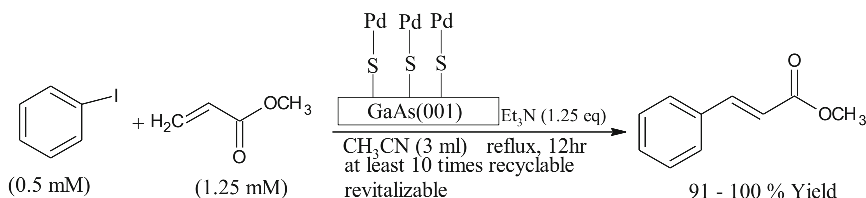
Nowadays, the basic problem in drug discovery is the increasing rate of failure in clinical trials, due to the problems associated with inappropriate pharmacokinetics, poor efficiency, and high toxicity. However, different strategies have been adopted to minimize the risk of failure in drug discovery. To find a better drug profile, it is important to determine the absorption, distribution, metabolism, elimination (ADME), and toxicity pattern as well as activity and selectivity of the drug molecule. To accelerate the drug discovery, significant improvements have been made by adding few novel techniques such as polymer-assisted solution-phase synthesis

(PASPS), microwave-assisted organic synthesis (MAOS), continuous flow process as well as ultra-performance liquid chromatography (UPLC), and supercritical fluid chromatography (SFC) for the improvement of an analytical profile of the drug molecule [53].

8.6 Role of Catalysts

Currently, the fundamental development in organic chemistry is organometallic catalyzed carbon–carbon bond formation and its application in medicinal and process chemistry. The discovery of two novel organometallic catalysts is ruthenium hydride with a nitrogen-containing heterocyclic carbine (Eq. 8.6) [54] and organopalladium catalyst supported on a sulfur-terminated semi-conductor gallium arsenide (001) (Eq. 8.7) [55]. The most important factor is the benign nature of both the catalyst as they generate indole derivative with sufficient atom density and increases catalysis by tenfold in Mizoroki–Heck reaction with an only trace amount of leached palladium. Ruthenium carbine catalysts used in the synthesis of biologically active natural products, due to their stability, functional group tolerance, easy handling, and commercial availability. Identification of the real catalyst is one of the critical elements in the development of a novel heterogeneous catalyst [56].





8.6.1 Catalytic Role of Metal Ions

The most important and innovative catalytic role of Zn-dependent histone deacetylase 8 (HDAC8) is to remove acetyl moieties from histone tails, which generally regulate transcriptional repression and gene silencing due to the closed chromatin structure. The catalytic process of Zn-dependent HDAC8 is of high importance as it shows novel development in antineoplastic agents. Previously in 2006, FDA (Food and Drug Administration) accepted SAHA (suberoyl + anilide + hydroxamic acid) as an anticancer drug which is directly combined with catalytic Zn ion and HDAC inhibitor [57].

8.6.2 Organometallics Catalysis

Organometallic enzymes play a pivotal role in drug discovery and development. The natural enzyme (hydrogenase) and artificial enzyme (metalloenzyme) represent the novel innovation in drug discovery [54]. The inhibition of carbonic anhydrase metalloenzyme plays a pivotal role in the development of anti-cancerous, anticonvulsant, and anti-glaucoma drugs. Metalloenzyme carbonic anhydrase also possesses the potential to develop anti-infective drugs (anti-fungal and anti-bacterial agents) [55]. Among solid catalysts, metal oxides have been considered as the most important as well as extensively utilized category. Mixed metal oxides represent mainly in heterogeneous catalysis and widely employed in the academic research, chemical, and pharmaceutical industries. Transition metals, among the metal oxide catalysts, played an important role extensively due to several advantages, i.e., selective action, easy regeneration, and low cost of production. They are used in different important reactions which include isomerization, oxidation, dehydration and dehydrogenation, etc.

The mixed metal oxides are widely used in different fields, especially in catalysis and organic synthesis. Mixed metal oxides played a vital role in catalysis and in the field of organic synthesis with the fundamental application in drug discovery [58]. Metal salts (e.g., CuCl, $AuCl_3$, $Pd(OAc)_2$) have been widely used for the catalytic processes that eventually discover many different reactions and become a part of organic synthesis. This leads to the formation of catalysts with improved reactivity, selectivity, high level of purity, and a minimal amount of waste material. The tunability and modularity of ligand trait in catalyst functioning can be beneficial for the development of novel entities [59]. The two most impactful and transformative fields of chemistry are organometallic and catalytic chemistry. Drug discovery and material synthesis and its transformation are the major achievements of these disciplines. It has been found that catalysts containing transition metal complexes are the most successful candidates in the field of drug discovery and stability [60].

8.7 Factors Affecting Stability

There are many factors that affect the stability of the drug candidate. Instability may lead to the incorrect bioassay, erroneous SAR's, false-positive high-throughput screening (HTS) hits, low bioavailability, as well as drug withdrawal. Some of the factors which influence the stability of drug are briefly discussed below:

8.7.1 Effect of pH

pH plays a vital role in the stability of pH-dependent compounds. Some compounds are stable at neutral conditions whereas others are stable under acidic or basic conditions [25]. Different studies have been carried out on the effect of pH as a function of stability on different drugs (i.e., riboflavin (RF) [61], cyanocobalamin (CY) [62], levofloxacin (LV) [63], and moxifloxacin (MF) [64]. It has been found that the stability of the drug moiety is dependent on the ionic state (anion, zwitterion, cation) and susceptibility toward H^+/OH^--catalyzed hydrolysis.

8.7.2 Effect of Concentration

Degradation of certain compounds directly depends on the initial concentration of drug and with the interacting substance (counterion) present in the system. Stability can be determined with a variety of counter ions (i.e., excipients, buffer components, sensitizers, etc.). A suitable buffer at a particular pH should be selected to achieve optimum stability. Degradation rate can be increased with the higher counter ion concentration. Degradation of various drugs (i.e., cefaclor (CF) [65], RF [66–68], etc.) under the influence of buffer has also been reported in several studies.

8.7.3 Effect of Ionic Strength

The ionic strength of buffer can affect the stability of certain drugs i.e., RF [69], gonadorelin [70] and indomethacin [71]. It alters the pK_a and activity of coefficient (enthalpy change) which accelerates acid or base catalysis.

8.7.4 Effect of Co-solvents

Co-solvents may produce unwanted effects on stability by changing the dielectric constant of the medium which may lead to the degradation or enhanced stability of drugs (i.e., azathioprine (AZ) [72], RF [73], thymoquinone (TQ) [74], etc.).

8.7.5 Effects of Protein Binding

The addition of protein can reduce the rate of hydrolysis, owing to protein binding and reduction of free-drug concentration. Bound drug molecules RF [75], roscovitine [76], are not fully accessible to acid- or base-mediated hydrolysis. It has also been demonstrated that stability at pH 7.4 was improved with the addition of serum protein (4%) for a set of carbonate esters and phenylacetate [77, 78].

8.7.6 Effect of Light

Some drugs are sensitive to light (i.e., UV (180–380 nm), visible (380–780 nm)) and its exposure results in the enhanced degradation/decomposition (i.e., RF [61], cyanocobalamin [62], difloxacin (DF), and sarafloxacin (SF) [79]). Photosensitive drugs in aqueous medium or solid dosage form are susceptible to the degradation which results in the toxicity to biological system. Photodegradation reactions of drugs depend on the type of reaction initiated by light i.e., photooxidation (e.g., AA), photoreduction (e.g., RF), photoaquation (e.g., CY), photocyclization (e.g., meclofenamic acid (MA)), photodealkylation (e.g., chloroquine (CQ)), photodecarboxylation (e.g., amino acids), photoisomerization (e.g., aztreonam (AZ)), photodimerization (e.g., primaquine (PQ)), photo-induced hydrolysis (e.g., sulfacetamide (SF)), and photo-induced ring cleavage (e.g., norfloxacin (NF)).

8.7.7 Effect of Temperature

High temperature results in the increased collision between drug molecules in an aqueous medium that leads to the degradation/decomposition of drugs (i.e., RF [80], meropenem (MP) [81], glibenclamide (GA) [82], etc.). The rate constant for the degradation of drug in relation to temperature is expressed by the Arrhenius equation (Eq. 8.8).

$$k = \mathrm{A}e^{\frac{-\mathrm{E}\alpha}{k_{?}\mathrm{T}}} \tag{8.8}$$

8.7.8 Hydrolysis

The most common factor that influences drug stability is hydrolysis, especially in cases of drugs in aqueous medium or liquid formulations (i.e., aspirin (ASA) [83], RF [84], CP, florfenicol (FF), spiramycin (SM) and tylosin (TY) [85], etc.). Hydrolytic degradation of drugs is influenced by the pH of the medium.

8.7.9 Oxidation

Oxygen and oxidizing agents (i.e., H_2O_2, $KMnO_4$, etc.) leads to the oxidative degradation of drugs (i.e., AA [86], morphine (MO) [87], vitamin A [88]). When these drugs are exposed to oxygen during manufacturing or storage, their drug content is altered. The pH of the medium enhances the oxidative degradation of drugs due to the change in the redox potential of the species in the drug molecules.

8.7.10 Effect of Catalytic Agents on Drug Stability

There are different types of catalysts (i.e., organic and inorganic catalysts) which could affect the stability of drugs in different media (i.e., aqueous solution, polar and non-polar solvents). The effects of these catalysts on the stability of drugs are discussed below.

8.7.11 Inorganic Catalysts/Synthetic Catalysts

Metal ions (i.e., Cu^{2+}, Fe^{3+})- and metal chelates-catalyzed oxidation of ascorbic acid (AA) have been evaluated. The study has been carried out in the pH range of 2.0 to 5.5 where only monoionic species of AA were found to be reactive toward molecular oxygen ($^3O_2$). It has also been noticed that un-catalyzed oxidation of AA was proportional to the concentration of $^3O_2$. Furthermore, the direct oxidation of ascorbate anion (AH^-) has also been found to be influenced by $^3O_2$. The catalytic oxidation of AA caused by metal ions follows first-order kinetics depending on the concentration of $^3O_2$. In the case of Cu^{2+}-catalyzed oxidation of AA, the rate of oxidation increases with an increase in the pH from 1.5 to 3.5, whereas Fe^{3+}-catalyzed oxidation of AA increases from pH 1.50 to 3.0 and afterward decreases to pH 3.5. The difference in the catalytic activity of these metal ions (Cu^{2+}, Fe^{3+}) toward monoionic and neutral species of AA is due to the extent of the formation of metal complexes [89].

The effect of metal ions and chelating agents on the degradation of DNA by bleomycin (BM) has been carried out. In this study, the effect of reducing agents (i.e., 2-mercaptoethanol, dithiothreitol, reduced nicotinamide adenine dinucleotide phosphate, hydrogen peroxide, ascorbate) has been evaluated on the degradation of DNA by BM. It has been found that BM at a concentration of 10 μg/ml has a minimum effect on the degradation of DNA. However, at a higher concentration of BM (50 μg/ml), it decomposes DNA. In the presence of ethylenediaminetetraacetic acid (EDTA) (10^{-3} M), the degradation of DNA by BM was fully diminished. Deferoxamine is a strong and highly specific chelator of Fe^{3+} and has greater inhibition in the degradation of DNA in the presence of BM as compared to that of EDTA, whereas 2-mercaptoethanol enhanced the decomposition of DNA by BM and additionally Mg^{2+} ions along with 2-mercaptoethanol further accelerate its decomposition [90]. The effect of metal chelates (Fe^{3+}-EDTA) on the degradation

of hyaluronic acid has been carried out. It has been found that the degradation of HA was strongly inhibited by superoxide dismutase and catalase. However, in the presence of Fe^{3+} ions, degradation of HA via an autoxidation was weakly inhibited by catalase whereas unaffected in the presence of superoxide dismutase. Furthermore, penicillamine (1.0–5.0 mM) also enhanced the degradation of HA in the presence of Fe^{3+}-EDTA chelates [91].

Fosinopril (FP) is an angiotensin-converting enzyme inhibitor. Kinetics and mechanism of metal ions (i.e., Mg, Co, Mn, Zn, Ni, Cu, Fe, Ca, and Ba)-mediated degradation of FP in methanol have been determined. It has been found that these metal ions enhanced the degradation of FP. Rate constants (k) for the degradation of FP in the presence of metal ions have been determined with a decrease in the concentration of FP. The rate of degradation of FP in the presence of metal ions was in the order of $Co^{2+} > Mn^{2+} > Mg^{2+} > Zn^{2+} > Ni^{2+} > Mn^{3+} > Cu^{2+} > Fe^{2+} > Ca^{2+} > Ba^{2+}$. Electron paramagnetic resonance (EPR) spectroscopy has been used to identify the mechanism of metal ions-mediated degradation of FP and was due to the formation of FP-metal ion complex followed by deprotonation [92].

In the presence of RF (as sensitizer), Cu^{2+}-mediated degradation of calf thymus DNA and supercoiled pBR322 has been observed. It has been found that the rate of degradation of DNA was higher in the presence of Cu^{2+} and RF as compared to RF alone. The rate of reaction for the degradation of DNA has also been found to be dependent on the presence (aerobic condition) and absence (anaerobic condition) of oxygen. In anaerobic conditions, this reaction was inhibited. RF act as a reducer of Cu^{2+} which converts it into Cu^{1+} and this mechanism was confirmed by Cu^{1+} sequestering neocuproine reagent. Cu^{2+} concentration plays an important role in the degradation; therefore, with an increase in the concentration of Cu^{2+} from 5.0–25.0 μM, the percent degradation of DNA also increased from 21.5 to 40.6% [93].

Peptides methionine residues were converted into methionine sulfoxides due to the action of oxygen, metal (i.e., Fe^{3+}), and electron donors (i.e., ascorbic acid, dithiothreitol (DT)). A study has been carried out to inhibit the oxidation process at different pH in the presence of hydroxyl radical scavengers (superoxide dismutase) and azide. Superoxide dismutase has been found to possess minimum oxidative effect which indicates that superoxide radical does not have any effect on the methionine residue oxidation. In this study, the effect of Fe^{3+} combined with AA and DT on two peptides (HM and GGGMGGG) to affirm the generation of oxygen species. These two peptides were degraded in the presence of AA (2 mM)/ Fe^{3+} (0.02 mM) and DT (2.0 mM)/ Fe^{3+} (0.02 mM) and phosphate buffer (pH 7.0, 10 mM). The degradation kinetics of peptide was found to be dependent on the nature and activity of pro-oxidants. In the presence of AA/ Fe^{3+} and DTT/ Fe^{3+}, the degradation of HM follows zero-order and first-order kinetics, respectively [94]. Degradation kinetics of cefaclor (CF) (an antibiotic) in the presence of Cu^{2+} as a catalyst has been evaluated. Cu^{2+}-catalyzed hydrolysis of CF has been determined using UV-visible spectroscopy. It has been observed that Cu^{2+} slightly increased the degradation of CF in aqueous media, whereas it was markedly increased in the case of other cephalosporins. This slight increase in the rate of CF

hydrolysis was due to the interaction of Cu^{2+} with the side-chain or carboxyl group [65].

The effect of pH, Cu^{2+}, and EDTA on photolytic and oxidative degradation of antiemetic (RG12915 (RG1)) has been evaluated. It has previously been known that when RG1 is exposed to artificial fluorescent light, dichlorination and substitution occur in an aqueous medium. Transition metals are known to catalyze the autoxidation of hydrocarbons. It has been found that with the addition of Ni^{2+} and Fe^{2+} to the solution of RG1, there was no significant effect found on the auto-oxidation. However, when Cu^{2+} was added in the solution of RG1, the degradation rate was significantly increased. This increase in the rate of degradation of RG1 may be due to the initiation of an auto-oxidation reaction [95].

Zn^{2+}-based catalytic degradation of four penicillins including amoxicillin (AX), ampicillin (AP), penicillin G (PG), and V (PV) in methanol at 20 °C has been carried out. The kinetic scheme for the degradation of these drugs in the presence of Zn^{2+} has also been reported in this study. It has been found that PV degradation occurs with the formation of a single intermediate substrate–metal (SM) complex. However, other drugs degraded with the formation of the metallo complex (MC1 and MC2). This Zn^{2+}-catalyzed degradation of drugs follows first-order kinetics with the rate constants of 0.93, 2.88, 3.04, and 3.49×10^2 min^{-1} for AX, AP, PV, and PG, respectively [96].

Oxidative degradation of HA in oxidation system containing AH^-, Cu^{2+}, and H_2O_2 have been carried out in the presence and absence of drug-naproxen (NP) or acetylsalicylic acid (ASA). It has been found that the solution containing HA results in decrease viscosity, which is an indication of polymer degradation. By the addition of drug in HA solution, it has been found that the degradation of the polymer was inhibited. Fragmented/decomposed polymers were characterized using FTIR spectrometry, size exclusion chromatography (SEC), and high-performance liquid chromatography (HPLC) [97].

Spectrometric studies have been carried out to evaluate the extent of degradation kinetics of doxycycline (DX) in the presence of Cu^{2+} and H_2O_2. The degradation of DX follows pseudo-first-order kinetics. The effect of temperature, pH, Cu^{2+}, and H_2O_2 on the degradation kinetics has been determined. Reactions for the degradation have been carried out in the temperature range of 20–35 °C and there was no effect of temperature at 20–30 °C. However, at higher temperature, the reaction rate was accelerated at the initial time but afterward, it gets slower. The effect of pH (7.8–10.2) on the rate of degradation has also been carried out, and it has been found that at pH 8.6, the rate of degradation was higher whereas at pH 10.2 the rate was lower. The effect of Cu^{2+} ion and its concentration ($1.33–4.33 \times 10^{-5}$ M) on the degradation rate have also been determined. In the concentration range of $1.33–3.33 \times 10^{-5}$ M, the degradation rate follows pseudo-first-order kinetics, whereas at higher concentration, the degradation rate follows negative pseudo-first-order kinetics. However, with an increase in the concentration of Cu^{2+} rate of degradation increased. The degradation kinetics for the DX in the presence of H_2O_2 follows similar behavior as with Cu^{2+} ion. At the concentration of $1.00–2.00\times$

10^{-2} M, H_2O_2 follows pseudo-first-order kinetics, whereas at higher concentrations, it follows negative pseudo-first-order kinetics [98].

In the presence of ruthenium (Ru^{3+}), the catalytic oxidation of amitriptyline (AM) by potassium permanganate has been carried out at a constant ionic strength (0.20 mol/dm^3). In acidic medium, it has been found that the rate of catalytic oxidation was increased with an increase in acid concentration. The rate of catalytic oxidation was found to be eightfold higher in the presence of Ru^{3+} as compared to that of the uncatalyzed reaction. The effect of temperature on the Ru^{3+} base-catalyzed oxidation has also been determined, and it has been found that with an increase in temperature, the rate of Ru^{3+}-catalyzed oxidation of AM also increased [99].

Photodeposition technique has been used to prepare silver-doped TiO_2 (Ag-TiO_2) NPs and was characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), and energy dispersive X-ray (EDX) spectroscopic techniques. SEM and EDX analysis showed a dispersion of Ag metal on the surface of TiO_2. The photocatalytic property of these prepared NPs has been determined for the removal of chloramphenicol (CP) after UV irradiation. The effect of experimental parameters (i.e., doping concentration of Ag, the concentration of photocatalyst, calcination temperature) on the photocatalytic activity of on CP has been determined. It has been found that the photocatalytic activity of TiO_2 doped with Ag increases at the calcination temperature of 300 °C [100].

Analgesic drug (dipyrone (DP)) is degraded by hydrolysis to form 4-methylaminoantipyrine (4-MAA). Oxidation processes (Fenton (FP), photo-Fenton (PFP), UV/H_2O_2 photolysis (UVP), UV/TiO_2 photocatalysis (UPC)) have been carried out for the comparison of efficiency to remove 4-MAA from aqueous media. It has been found that the removal of 4-MAA through oxidation processes was 94.1, 96.4, 74.4, and 71.2% for FP, PFP, UCP, and UPVC, respectively. The removal of 4-AMM was due to the breakdown of three methyl moieties, followed by pyrazolone ring breakage leading to the formation of different intermediates. These intermediates were hydroxylated and carboxylic derivatives. The removal of 4-MAA has been carried out by varying concentrations of Fe^{2+} and H_2O_2 and pH from 1–3 mM and 5 to 25 mM and 2.0 to 4.0, respectively. The 4-MAA removal efficiency was enhanced with an increase in the concentration of Fe^{2+} and H_2O_2 and pH. The mechanism of degradation of DP is based on the formation of the oxidizing agent. However, the extent of degradation of DP and the formation of intermediates is based on the pH and concentration of Fe^{2+} and H_2O_2 [101].

TiO_2-SiO_2 composites with different concentrations of TiO_2 (10–40%, *w/w*) have been prepared using mesoporous NPs (MNPs) and characterized by N_2-physisorption, X-ray powder diffraction (XRPD), diffusive reflective UV-vis spectroscopy (DRUV-vis), X-ray photon spectroscopy (XPS), and TEM. The prepared composites were spherical with high surface area and titania was found in the anatase phase. The physical and chemical properties of these composites are specific for the catalytic process so, therefore, they were used for the photocatalytic degradation of methylene blue (MB), methyl orange (MO), and paracetamol (PM). The MNPs provide special characteristics to these composites, i.e., increase surface

area and thermal stability, and also modify their photocatalytic properties. It has also been found that loading on TiO_2, the particle size and surface characteristics influence the degree of UV absorption and energy bandgap between composites. Under UV irradiation, in the presence of TiO_2–SiO_2 composites, the concentration of MB and MO were found to decrease with the time. The photocatalytic activity of these prepare nanocomposites has been tested for the degradation of PM. It is a neutral compound that possesses a phenolic ring and has been found to adsorb on the surface of photocatalyst that results in the stabilization because of its neutral nature [102].

Photocatalytic degradation of chloramphenicol (CP) and tartrazine (TZ) has been carried out in an aqueous suspension of silver-modified TiO_2 (Ag/TiO_2) nanoparticles (NPs) after UV irradiation. These Ag/TiO_2 NPs were prepared by chemical reduction and were characterized using XRD, SEM, energy dispersive X-ray microanalysis (EDX), TEM, and XPS techniques. XPS measurements indicated that the Ag was present in the Ag^0 form on the surface of TiO_2 NPs. The effect of calcination temperature, Ag concentration, loading on photocatalyst, substrate initial concentration, and intensity of light has been evaluated on the photocatalytic degradation of CP. The results obtained after photocatalytic degradation of CP and TZ indicated with an increase in the concentration of Ag on TiO_2 NPs enhance degradation. However, the results obtained showed that the degradation of TZ (89%) was greater as compared to that of CP (84%) in these sets of experimental conditions [103]. One-pot simple synthetic method (green method) has been used to prepare doxycycline (DX)-stabilized silver nanocatalyst (DX-AgNPs) for the rapid catalytic degradation of ibuprofen (IB) and paracetamol (PM). These nanocatalysts were characterized by UV-vis spectrometry and TEM. UV-vis spectrometry of these nanocatalysts showed surface plasmon resonance (SPR) band at 404 nm. TEM analysis confirmed that the prepared nanocatalysts are spherical shaped and monodispersed with a particle size of 6.87 ± 2.2 nm. These nanocatalysts show excellent photocatalytic properties with 100% degradation of IB and PM in 1 min [104].

A photocatalytic (solar irradiation) degradation study on anti-inflammatory (ibuprofen (IB)) drug has been carried out using transition metals (Bi and Ni) doped TiO_2. Nanoparticles (NPs) of TiO_2 doped with Bi and Ni have been prepared using sol–gel method and were characterized using XRD, SEM, UV-vis reflectance spectroscopy, and Brunauer–Emmett–Teller (BET) analysis. These catalysts (metal-doped NPs) were synthesized by different concentrations (0.25–1.0, *w*/*w*) of the doped materials. IB was used as a model compound to evaluate the photocatalytic property of these doped NPs and compared with the Degussa TiO_2 to evaluate the kinetics of the drug degradation. Bi-doped TiO_2 NPs showed higher photocatalytic degradation of IB as compared to that of Ni-doped TiO_2 NPs or Degussa NPs. IB decomposition was found to be 89 and 78% after irradiation with Bi-doped TiO_2 and Ni-doped TiO_2 NPs, respectively. Kinetics of degradation of IB in these experimental conditions has been determined using the Langmuir–Hinshelwood model and found that its degradation follows first-order kinetics with

the degradation rate (*k*) of 6.40 and 4.60 $\times$ 10^{-3} min^{-1} for Bi and Ni-doped TiO_2 NPs-mediated photocatalysis, respectively [105].

Photocatalytic activity of semiconductors could be increased by doping them with rare earth metals nanoparticles (NPs). The gel combustion method has been used to prepare zinc oxide (ZnO) and lanthanum (La)-doped ZnO NPs. It has been found that when La is used as doping material of ZnO NPs, the cytotoxicity and photocatalytic activity were enhanced toward difference cell lines and paracetamol (PM) drug. These prepared doped ZnO NPs were characterized by UV-vis spectroscopy, SEM, TEM, XRD, and FTIR spectrometry. The spectroscopic studies showed that La-doped ZnO NPs exhibited absorption maxima in the visible region due to the presence of La [106].

Bimetallic (Fe and Ni) NPs have been synthesized and were characterized by SEM, energy-EDS, and XRD analysis. These bimetallic NPs were found to be in the particle size range of 20–200 nm and were used to determine their reductive degradation effect on nimesulide (NM). SEM confirmed that the prepared NPs are uniformly bound with silica and XRD analysis showed the presence of Ni and Fe on silica. Reductive degradation effect on NM was evaluated using bimetallic NPs containing Ni (8%, *w/w*) at an agitation frequency of 250 rpm. The reductive degradation products after degradation were determined using liquid chromatography-coupled electron spray ionization mass spectrometry (LC-ESI-MS). This study has confirmed that the degradation of NM mainly occurs through the reduction of the nitro group and the elimination of sulphonyl moiety. This reductive degradation of NM leads to the formation of an amine and thioester aromatic as byproducts [107].

The sol–gel method has been used to prepare carbon-doped WO_3/TiO_2 photocatalysts. These prepared photocatalysts were characterized using XRD, diffuse reflectance UV-vis spectroscopy, nitrogen adsorption-desorption analysis, SEM, TEM, and XPS. The photocatalytic activity of these modified mixed oxides has been evaluated for the degradation of sodium diclofenac (SDF) by solar irradiation. High-performance liquid chromatography (HPLC), total organic carbon (TOC) analysis, and ion chromatography (IC) have been used to evaluate the degradation and mineralization of SDF. Tungsten and carbon (TWC)-modified photocatalyst exhibits higher activity as compared to that of TiO_2 and WO_3/TiO_2 for the degradation SDF [108].

A mechanistic study and degradation pathway for the photocatalytic degradation of fluoroquinolone (levofloxacin (LF)) using quantum dots (QDs) (Ag_2O/TiO_2) has been carried out. The QDs were prepared using a pH-mediated precipitation method and were characterized for their morphology, composition, structure, optical, and photocatalytic properties. Morphological studies confirmed the formation of QDs with a particle size of 2–9 nm. XPS analysis for surface indicates that the Ag^+ and Ti^{+4} were present in the form of Ag_2O and TiO_2 on the surface of QDs. Spectroscopic properties reveal that the absorption maxima of Ag/TiO_2 QDs shift to the longer wavelength (506 nm) as compared to TiO_2 QDs (394 nm). It has been found that these QDs possess excellent photocatalytic properties for the degradation of LF as compared to that of TiO_2 QDs. This excellent photocatalytic property was

attributed to the electron-hole pair separation that results in the higher absorption of light [109].

The study has been carried out to evaluate the metal ion (Fe^{3+})-catalyzed oxidative degradation of some aromatic drugs i.e., dextromethorphan (DM), epinastine (EP), brodimoprim (BD)/drug fragment that possesses C-H bonds in the absence of peroxides. Fe^{3+} is known to oxidize peroxides to form peroxy and alkoxy radicals which oxidize drug molecules. Transition metals are known to oxidize electron-rich centers of aromatic rings which results in the formation of aromatic radical cations. This aromatic radical cation, when attached to the benzylic side-chain, will overlap the σ-orbital with the hydrogen of the benzylic ring system and leads to the breakdown of the C-H bond. H atom from the C-H bond is then transferred to the aromatic ring, which leads to the formation of benzylic radical. This benzylic radical in an oxygen-saturated system react with oxygen to form peroxy radical. This peroxy radical follow the Russell mechanism, which results in the formation of alcoholic and ketonic degradation products [110].

Spectroscopic and thermal degradation of Mg^{2+}, Ca^{2+}, Ba^{2+}, and Sr^{2+} of PM has been carried out. Complexes of Mg^{2+}, Ca^{2+}, Ba^{2+}, and Sr^{2+} of PM have been prepared and characterized by elemental analysis, thermogravimetric analysis (TGA), conductivity, UV-vis spectrometry, IR, and ^1H NMR spectroscopy. Molar conductivity values for these metal complexes were found to be in the range of 57.4 to 72.8 $cm^2\ mol^{-1}$. The microanalytical analysis revealed that there was a 2:1 molar ratio complex between PM and metal ions [111]. The microwave hydrothermal process has been used to prepare Ag-dispersed $BaMoO_4$ octahedron microcrystals doped with Er^{3+}, Yb^{3+}, and K^+. These doped microcrystals were characterized by XRD, XPS, UV-vis diffuse reflectance spectrometer, Raman spectroscopy, and field emission scanning electron microscope (FESEM). The photocatalytic activity of these prepared microcrystals for the degradation of rhodamine B (Rh B) and IB after solar and visible irradiation, respectively, has been determined. In the presence of these doped (Er^{3+}/Yb^{3+}) microcrystals, the degradation of Rh B and IB was around 99.60 and 41.50% in 90 min, respectively. Degradation products after irradiation of IB in the presence of doped microcrystals have been identified by high-resolution quadruple-time of flight-electrospray ionization mass spectrometry (HR-QTOF ESI/MS) in negative ion mode [112].

The effect of metal ions (monovalent, divalent, and trivalent) on the degradation of riboflavin (RF) has been evaluated in aqueous media. This study has been carried out in low (0.2 M) and high (0.4 M) phosphate buffer concentration at pH 7.0. These metal ions include Ag^+, Zn^{2+}, Mg^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, and Fe^{3+}. RF (5.0×10^{-5} M) was photolyzed at pH 7.0 with these metal ions ($1.0–5.0 \times 10^{-4}$ M), and it was found that with an increase in the concentration of metal ions, the rate of photolysis also increases. It has been found that except Ag + ions, all other metal ions accelerate the degradation of RF. The second-order rate constants for the interaction of these metal ions with RF are in the order of $Zn^{2+} > Mg^{2+} > Pb^{2+} > Mn^{2+} > Cu^{2+} > Cd^{2+} > Fe^{2+} > Ca^{2+} > Fe^{3+} > Co^{2+} > Ni^{2+} > Ag^+$. These metal ions form a metal-RF complex in the ground state which on irradiation forms exciplex due to charge transfer which leads to the formation of

semiquinone radical and oxidized metal ion. This semiquinone radical combine with another semiquinone radical to form reduced RF which was then oxidized to give degradation products [113].

ZnO NPs for nanocatalysis has been prepared for the catalytic degradation of amlodipine besylate (AMB). These prepared NPs were characterized using SEM, XRD, FTIR, BET, Barrett-Joyner-Halenda (BJH), EDS, X-ray fluorescence (XRF), and UV-vis spectrometry. Different degradation parameters (i.e., initial concentration, nanocatalyst concentration, pH, temperature, time of visible irradiation, H_2O_2) were used to evaluate the parameters dependent on degradation of AMB. The initial concentration of analyte plays an important role in the rate of degradation; therefore, it has been found that at higher concentrations of AMB, the catalytic efficiency of these NPs was reduced due to the absorption of AMB on the surface of these NPs. The photocatalytic activity of ZnO NPs was found to be maximum at 3 mg whereas at higher concentrations of nanocatalysts, the photocatalytic activity would not be observed. Previously it has been reported that AMB degraded more rapidly in the alkaline region as compared to that of the acidic region. However, these nanocatalyst dissolved in a highly acidic medium, so for this reason, Fe^{2+}-doped ZnO NPs are the best catalyst for the degradation of AMB at all pH range. The rate of reaction was temperature dependent, and at a higher temperature (80 °C), nanocatalyst accelerates the degradation of AMB [114].

8.7.12 Organic Catalysts/Natural Catalysts

Chondroitin sulfate (CS) is a naturally occurring polymer containing glucuronic acid and N-acetyl glucosamine. CS is sulfated at one position (either 4 or 6) and is used for the treatment of osteoarthritis. The stability of CS has been determined in the presence of tissues and lumenal components of the stomach, small intestine, cecum, and colon. It has been found that the components of tissues, stomach, and small intestine do not degrade CS, whereas the components of cecum and colon promoted its degradation. ^{14}C-radiolabelled CS has been used to determine the degradation of CS and its degradation products (disaccharides). In vitro degradation of CS has been carried out in the presence of commercially available chondroitinase which is determined using the spectrometric method. This method has confirmed that in the presence of chondroitinase, the degradation of CS has been around 80%, whereas in the presence of stomach and intestinal components, no degradation has been found. In vitro transport mechanism of CS across the gastrointestinal tract has also been determined using ^{14}C-radiolabelled CS and found that CS transportation across the small intestine was via endocytosis [115].

Glutathione S-transferase (GSTs) enzymes have been used to evaluate the degradation of some antibiotics including tetracycline (TC), sulfathiazole (SF), and ampicillin (AM). GSTs are the proteins that catalyze the conjugated system of reduced glutathione in the presence of hydrophobic substances having electrophilic centers. In the presence of GSTs, these antibiotics are converted into non-toxic

byproducts in the range of 30 to 70%. The conversion of these antibiotics in byproducts was 30% for TC and 60–70% for SF and AM [116].

Catalytic degradation of amygdalin (AMD) with enzyme from *Aspergillus niger* has been carried out. AMD was a controversial drug that has been used as an anti-cancerous agent for many years. It has previously been found that AMD itself does not possess any anti-tumor activity, but its degradation products might possess anti-cancerous activity. AMD was exposed to the extracellular enzyme from *Aspergillus niger* for catalytic degradation at 37 °C and found that in 4 h, it was degraded into four degradation products. These four degradation products are then isolated and purified using chromatography and further characterized by mass spectrometry, ^{13}C, and ^{1}H NMR. These products have been identified as mandelonitrile, prunasin, phenyl-(3,4,5-trihydroxy-6-methyl-tetrathydro-pyran-2-yloxyl)-acetonitrile (PTMT) (a novel hydroxy derivative of prunasin), and benzaldehyde. This novel hydroxyl derivative (PTMT) has been found to inhibit the growth of S-18 tumor cells in 11 days, depending on the concentration of PTMT [117].

A study has been carried out on the effect of enzymes isolated from *Planococcus* sp. S5 (gram-positive bacteria) on the degradation of naproxen (non-steroidal inflammatory drug (NSAID)) (NX). It has been found that in the presence of these enzymes, the degradation was around 27%. The influence of growth substrates (i.e., benzoate, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, vanillic acid) on the degradation of NX has also been evaluated. It has been found that the degradation of NX was around 21.5, 71.7, 14.7, and 8.2% in the presence of benzoate, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and vanillic acid, respectively. Furthermore, it was noticed that *Planococcus* sp. S5 possesses an excellent ability for the degradation of NX in the presence of 4-hydroxybenzoate (carbon source) [118].

White-rot fungus *Pleurotus djamor* has been used to evaluate the degradation kinetics of NSAIDs (i.e., NX, diclofenac (DF), ketoprofen (KP)) in pure, mixtures, and submerged cultures. It has been found that DF alone and in the mixture (NX and KP) the removal was around 93 and 99%, respectively, in the presence of fungus after 6 h of incubation. After 48 h of incubation with fungus, the removal of NX and KP was around 90 and 87%, respectively. The catalytic activities of enzyme laccases, manganese, peroxidases, and lignin peroxidases on the degradation kinetics of NSAIDs have also been carried out. The catalytic activity of extracellular laccases increases in the range of 200–300% for the degradation of NSAIDs in submerged cultures. However, in the case of manganese peroxides, the activity was increased to 126 and 138% for DF and other NSAIDs, respectively. The catalytic activity of lignin peroxides for the degradation of NSAIDs in the mixture was around 23% [119].

8.8 Conclusion

Catalytic agents are specific for different chemical/biological reactions, and their use in different processes is an important attribute to enhance the processes rather than to destroy the final product or the formation of unwanted products. Catalytic reactions

are useful as they lead to the formation of different products which sometimes may be beneficial for the treatment of different diseases. However, these reactions may also sometimes cause toxic effects on the biological system and alter normal physiological functions.

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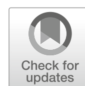

9 Analytical Techniques for the Assessment of Drug Stability

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Abstract

The expansion of the medications fetched an upheaval in the human healthcare system. These medicines would assist their role only when they do not have any contamination and being administered in the desired quantity. Stability studies are an essential part of drug development and crucial throughout all stages, with a precise timeline of analytical testing. Drug stability can be categorized into different types, including physical, chemical, microbiological, therapeutical, and toxicological stability. Different instrumental and chemical methods were devised for the analysis of drugs at regular intervals. There is always a risk of contamination in these drugs during different phases of their manufacturing, storage, and transportation, making them unsuitable for administration so they

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_9

should be identified and quantified. Various analytical techniques and instrumentations are playing a vital role for this purpose. In this chapter, we have discussed various analytical techniques and methodologies for the assessment of the drug's quality and stability. The chapter highlights a range of analytical methods, i.e., chromatographic, electrochemical, electrophoretic, spectroscopic, and titrimetric, and their analogous techniques that have been utilized in the analysis of drugs.

Keywords

Drug stability · Chemical kinetics · Types of drug stabilities · Analytical techniques

9.1 Introduction

Drugs and pharmaceutical product's stability is an interesting subject for pharmacists, regulators, and drug manufacturers. Stability is a key quality feature, and the degree of Good Manufacturing Practice (GMP) has an essential role in the drug development process. Among all of these drug properties, this is crucial because any change occurred due to physical or chemical processes over time may affect its quality, safety, and efficacy. In most countries, stability is a regulatory prerequisite for drug registration. This is compulsory to ensure that patients have access to safe and effective products throughout the shelf life. Most of the drugs can be sensitive to environmental factors that may cause changes in the physical state or chemical structure, so it is prone to physical, chemical, and microbial degradation in different environments. This can have severe effects on its biological safety and efficacy. For manufacturers, product quality is a key element during manufacturing, shifting, and storage.

Knowledge about the stability of a drug is required to select the appropriate materials for packaging and storage conditions to evade physical and chemical alterations and interactions among drugs and excipients. The medicines contained in the pharmacopeia need to be stored under prescribed conditions to preserve quality characteristics during the shelf life. In adverse climatic conditions such as high temperature and humidity, precautions must be taken to store medicines. Stability concerns are significant in developing therapeutically effective dosage forms. Manufacturers need to perform stability studies on all final products, comprising products that are diluted or reconstituted with 5% glucose solution/saline solution before use [1].

From drug discovery and biopharmaceuticals to drug supply and pharmaceutical technology, advances in all parts of pharmaceutical science have been closely related to the development and application of analytical technologies. Without the development of HPLC and its application in plasma samples, there would be no time curve of plasma concentration, nor would the concept of bioavailability be developed. Without solid-state analysis methods (such as thermal analysis and X-ray powder diffraction), it is impossible to study the significance of the solid-state phase of a

Table 9.1 Fraction of different analytical methods approved for the assay of bulk drug materials in USP XXVII and European Pharmacopoeia, fourth edition (Ph. Eur. 4)

Analytical methods	USP 27 (%)	Ph. Eur. 4 (%)
Non-aqueous	24	36.5
Potentiometric	10	27
Indicator	14	9.5
Aqueous mixtures	5.5	21
Potentiometric	1	14.5
Indicator	4.5	6.5
Titration	40.5	69.5
GC	2.5	2
Acid base	29.5	57.5
Microbiological test, e.g., antibiotics	2.5	3
HPLC	44	15.5
UV-vis spectrophotometery	8.5	9.5
Other, i.e., argentometry, complexometry, etc.	5.5	5.5
Redox, i.e., nitritometry, lodometery, etc.	5.5	6.5
Other, e.g., NMR, IR, fluorimetry, atomic absorption, polarimetry, polarography, spectroscopy, gravimetery, etc.	2	0.5

Adapted from Ref. [2]

drug on its drug performance, let alone understand, just two examples. This list may continue infinitely. However, as the problem of drugs has been clarified, analytical techniques have also advanced. For instance, the continuous development of dissolution testing, which was initially used for quality control of dosage forms, now also has physiologically related dissolution tests to comprehend the in vivo performance of dosage forms.

In the pharmaceutical research field, analysis of major APIs, intermediates, pharmaceutical products, pharmaceutical preparations, impurities/degradation products, biological samples comprising drugs, and drug metabolites is essential. Beginning with the prescribed pharmaceutical examination, analytical methods are mentioned in the pharmacopeias and monographs aiming at describing the drug substance's quality by limiting their content of active ingredient. Recently, assay techniques in the monographs cover titration, capillary electrophoresis, chromatography, spectroscopy, and electroanalytical methods. The current state of the art is represented in the Table 9.1 which is based on the US and European pharmacopeias [2].

Analytical technology plays an essential role at all drug stages during its development from sending to market and even after its marketing because a drug's stability about its physical and chemical properties affects the design and selection of a dosage form. It also evaluates the stability of a drug product, and quantifies when applicable, and evaluates drug content and impurities in a commercial product, and also those impurities above a predetermined threshold are identified, which are necessary to assess the toxicity profile of these impurities to differentiate them from

API. The analysis of drugs and their metabolites may be qualitative or quantitative and is widely used in pharmacokinetic research. This chapter highlights the stability study, its types, and importance as well as the role of several analytical techniques and methods in drug analysis.

9.2 Drug Stability

Drug stability means “a limit to which drug particle or a product can keep its integrity, within the specific time and the period through which it can be stored and further used, with the similar characteristics and features possessing at the stage of manufacturing”. The type of stability is usually divided into physical, chemical, therapeutic, toxicological, and microbiological. Drug stability can also be classified as a period before the product’s availability in the market. Pre-market stability approaches the clinical path where drug products can save under different strategies for safety and the ability to produce a desired or intended result. It is organized throughout the clinical examination in the drug filling duration. Commercial stability is progressive certainty on post-approval and the long-term stability examination of the drug product [3].

9.3 Importance of Drug Stability Studies

Stability studies are an essential part of drug development throughout all the stages with a precise timeline of analytical testing. The critical aim of stability testing is related to the patent’s well-being, which is suffering from the disease for which the product is being designed. Aside from the degradation of non-stable products into toxic disintegration products, up to 85% activity loss that is claimed on the label may cause therapeutic failure leading to death, i.e., nitroglycerine tablets for angina and cardiac arrest. Due to this concern, it becomes a legal prerequisite to provide statistics for particular stability testing for drug regulatory authorities for new drug approval [4]. The second main concern is protecting the manufacturer’s reputation by declaring the product’s suitability for use, given all functionally related qualities as far as they are in the market. An additional benefit of conducting product stability studies during the development phase or on the market is the provision of a database that might be valuable in selecting appropriate formulations, excipients, and container sealing systems for the development of new products to regulate the shelf life of the product and storage conditions for developing a new product, to prepare registration documents to verify the shelf life of the required registration documents, and verifying that no alterations have been induced in the formulation/manufacturing procedure that could severely affect product stability [5].

9.4 Types of Drug Stabilities

9.4.1 Physical Stability

Drug's physical stability focuses on the physical alterations that occur in the product. These changes subject to the physical characteristics of the drug, i.e., morphology, melting point, polymorphic texture behavior, and particle size. Liquid dosage forms' physical stability is affected due to alteration in appearance, discoloration, viscosity, precipitation, drug adsorption (container surface), polymorph formation (low solubility), and microbial growth. Variations in solid dosage form's physical stability include salt or salt exchange, polymorphic transformation, solvation/desolvation, moisture adsorption, amorphization, and then recovery to crystalline form. Such alterations may cause the physical instability of the product.

9.4.2 Chemical Stability

Chemical reactions that drugs undergo in a liquid dosage form which affects product stability include oxidation (ascorbic acid, epinephrine, vitamin A), epimerization (moxalactam, tetracycline, etoposide), dehydration (glucose, prostaglandins E1 and E2, erythromycin), hydrolysis (amides, esters, imides), isomerization (cyclosporine A, cytarabine, amphotericin B), decarboxylation (etodolac, 4-aminosalicylic acid), and so on. Screening for potential toxicity of degradation products is part of the safety assessment program. Now computer-aided technology is employed to predict the toxicological performance of drug disintegration products [6].

9.4.3 Microbiological Stability

Drug's microbial stability is critical for the safety and efficiency of the product. Resistance against microbial growth or sterility must be preserved throughout the shelf life. The effectiveness of the preservatives must stay unchanged within a specified range. Multi-dose liquid formulations comprise preservatives to prevent deterioration during use. Preservatives do not affect the susceptibility of a product for its contamination (that is, the process by which microorganisms enter the product depends mainly on the design of the container). However, good design can minimize the levels of organisms entered during use and can work in concert with active preservatives to safeguard consumers. Pathogen-contaminated products can have serious concerns for consumers and manufacturers, so the product largely relies on sufficient activity of a preservative. In order to obtain approval of regulatory authorities, it is essential to demonstrate sufficient preservative activity at manufacturing time and during shelf life. Hodges discusses the biological evaluation prerequisites for preservation activity [7].

9.4.4 Therapeutic Stability

The therapeutic effect will stay unchanged.

9.4.5 Toxicological Stability

No notable increase in toxicity occurs [8, 9].

9.5 Types of Stability Studies

Stability studies are based on checking the drug product for a long duration in different temperature and atmospheric moisture. Stability studies are mainly of four types:

9.5.1 Long Term Stability

If the drug is to be divided into many geographical areas and if shipping is the demand for shipment, in these kinds of situations, long-term stability studies are of great value. These studies are performed by checking the sample at a particular duration, and conditions of external variables are changed accordingly. The primary motive of this study is to measure the shelf life of the drug substance.

9.5.2 Intermediate Stability

Studies carried out at 65% RH and 30 °C and intended to reasonably elevate the chemical degradation rate or physical alterations for a drug product/substance proposed for long-term storage at 25 °C.

9.5.3 Accelerated Testing

These studies consist of overstating storage conditions designed to study the increased rate of bodily and chemical degradation. This is part of the formal stability studies. Data from these studies is used to carry out long-term stability studies, i.e., to examine the shelf life of the drug product.

9.5.4 In-Use Stability

This kind of stability study is actually for unit-dose or multi-dose drugs. The physical stability and chemical mixture of such drugs are such that due to continual

Table 9.2 Storage conditions for stability studies of pharmaceutical products

Storage condition	Stability study method	WHO test temperature and humidity	ICH test temperature and humidity
Freezer	Long term	−20 °C ± 5 °C	−20 °C/ambient (12 months)
Refrigerated	Long term	5 ± 3 °C	5 °C/ambient [12]
	Accelerated	30 ± 2 °C/65 ± 5% RH or 25 ± 2 °C/ 60 ± 5% RH	25 ± 2 °C/ 60 ± 5% RH (6 months)
Room temperature	Accelerated	40 ± 2 °C/75 ± 5% RH (6 months)	40 ± 2 °C/ 75 ± 5% RH (6 months)
	Intermediate	30 ± 2 °C/65 ± 5% RH (6 months)	30 ± 2 °C/ 65 ± 5% RH (6 months)
	Long term	25 ± 2 °C/60 ± 5% RH or 30 ± 2 °C/ 75 ± 5% RH (12 months) 30 ± 2 °C/ 65 ± 5%RH	25 ± 2 °C/ 60 ± 5% RH (12 months)

Adapted from Ref. [10, 11]

opening and closing, it gets deteriorate due to microbial impurity. The objective of in-use stability testing is to generate where applicable an in-time limit during which a multi-dose product can be used to keep possession of quality in an accepted specification once the container is opened [9]. The storage conditions for the stability studies of the pharmaceutical products are mentioned in Table 9.2 [10, 11].

9.5.5 Role of the Analytical Techniques in Drug Stability

In the field of pharmaceutical research, analysis of so many drugs, intermediates, raw materials, impure products, and biological materials that have drug products and drug metabolites is necessary. Since the initial stage of the authorized pharmaceutical assessment, assay procedures were mentioned in standard monographs to characterize the standard of a large number of drug materials by limiting the actual drug content. Recently, the assay procedures in monographs consist of spectrometry, titrimetric, chromatography, and capillary electrophoresis.

Electroanalytical techniques are also mentioned in the literature. The current stage-of-the-art is depicted by the data provided in Table 9.1 taken from US and European pharmacopeias [2]. From phases of drug growth to retail and post-retail, analytical methodology shows a significant role, considering the chemical and physical firmness of the drug, effects on the selection and dosage design, checking the firmness of the drug particles, quantization of contaminants, and recognition of those contaminants that are more than the organized threshold necessary for the assessment of toxicity profiles of these contaminants to eminent those from the API,

when validated and checked marketed drug product content. The assessment of drug and its metabolite, which could be qualitative or quantitative, is widely useful in pharmacokinetic studies [12].

9.6 Role of Chemical Kinetics in Drug Stability Studies

Chemical kinetics refers to the quantitative study of the chemical reaction rates, including factors that are affecting them. Drug stability study includes the kinetics assessment of chemical degradation reactions occurring in the drug dosage forms. It is essential to envisage the products' shelf life under distinct conditions of drug storage. The drug products can have many chemical structures and thus undergo various degradation modes having different order of reaction under different conditions. The frequently observed drugs' degradation reactions contain hydrolysis, oxidation, and photolysis. Mentioned degradation reactions mainly arise during storage, manufacturing, and usage of the drug products. The working pharmacists must be mindful of the outcomes of such reactions as a toxicity development or loss of potency in drug products to warrant the well-being of the patient. A precise evaluation of the potency loss of a product could be established through employing stability-indicating evaluation techniques which can also asses the degradants and other related compounds. Analyzed data are then passed through kinetic handling for assessing shelf life of drug products and for fixation of the expiry date. A reassessment is required in the packaging and storage parameters for any variation in dosage parameters to enhance drug stability. For this purpose, the reestablishment of the shelf life or reset phase under recommended storage conditions is required. Detailed information on the basic principles of chemical kinetics is required for the evaluation of the rates of drug degradation reaction and the estimation of shelf life and expiry date of the drug substance. These kinetic parameters can be utilized for interpretation of the degradation reactions' mechanisms enabling them to select suitable methods for drug stability. Numerous outstanding aspects of this topic with implications to the drug products' degradation kinetics are mentioned in the books [7, 13–19] monographs, [7, 20–24], and reviews [24–30].

9.7 Analytical Techniques

An analytical dimension is organized to check the limited properties of the drug material or drug outcome against traditional acceptance criteria for that property. Initial in designing a new analytical technique selection of the analytical methodology and the instrumentation that should be chosen exists on the proposed function and the capacity of the analytical technique [31]. Functions that may be assessed through the way events are care, diameters, the ceiling of observation, and limits of quantization (LOQ), range, sureness, and exactness [32].

9.7.1 Titrimetric Techniques

The procedure of titrimetric analysis originated in the eighteenth century. The term titration was originated from the invention of the volumetric method by Gay-Lussac in 1835. The assay method as being an old technique still has some advantages during recent times, such as an expansion of the non-aqueous titration technique, intensifying the area of applying these procedures to weak acids and bases and also by improving the endpoint of potentiometry to enhance the accuracy of these methods. Titrimetric methods have also gained the importance of kinetic measurements used to establish reaction rates due to the organization of functional group analysis procedures. These methodologies can be beneficial in saving time, labor, reasonable accuracy rate, and minimizing the use of reference points. In old times, titrimetric methods were used commercially for the dosage determination of albendazole [33], captopril [34], and gabapentin [2]. An antibiotic named sparfloxacin was also examined by the non-aqueous titration method [35]. These methods are mostly applicable for drug assessment but have also been used in pharmaceuticals for the assessment of the impurity of products [36].

9.7.2 Chromatography Techniques

9.7.2.1 Column Chromatography

The method of analyzing and separating chemical compounds from a mixture is known as chromatography, and the use of column chromatography can significantly purify a large number of such compounds. Column chromatography is a separation technology which is used in several fields, among which pharmacy is one of which pharmaceuticals are prepared and distributed based on this technology to warrant the effectiveness of drugs [37]. Column chromatography includes different types of columns, like flash columns, gravity columns, low- and medium-pressure columns, high-pressure columns, and vacuum columns. However, all of these columns are similar in that all columns need an absorbent, acting as a stationary phase through which samples comprising diverse compounds flow at different rates [38]. On the contrary, the adsorbent is subjected to the column in two ways, namely, a dry packing method and a slurry packing method. The slurry filling method is usually used for macro-separation, while the dry filling method is used for micro-separation. The chief advantage of column chromatography is that the stationary phase used in the method is comparatively inexpensive, and its handling is also easy. The latter stops cross-contamination and degradation of the stationary phase because of recycling. Column chromatography could be performed by gravity to move the solvent or use compressed gas to drive the solvent through the column [39].

Column chromatography can be employed in the purification of the reaction mixture during chemical synthesis, e.g., preferred alcohol and β-ketoester were refined from traces of sulfur, purification of polychlorinated biphenyls and silver nitrate filled silica gel, and purification of phenols, pesticides, organ chlorine, and polynuclear aromatic compounds. It is also used for the purification of biomolecules,

i.e., proteins, for example, the purification of pramlintide (peptide hormone) synthesis for the treatment of type 1 and 2 diabetes. Similarly, bioactive glycolipid's purification is also done by column chromatography, which is used for the treatment of herpesvirus (HSV-1). Also, the nucleic acid purification in cultures (in vitro and in vivo) is done by employing silica gel as an absorbent as it can absorb nucleic acid [40].

9.7.2.2 Gas Chromatography

GC is usually used for the vaporous organic compounds for their detection [41]. The combination of separation and on-border detection gives an exact quantitative organization of compound mixtures, inclusive of minute quantities of amalgam (parts/trillions in specific cases) [42]. GC plays a crucial role in pharmaceutical product analysis and formation of the high-molecular mass outcome, i.e., polypeptides/melting non-stable antibiotics. The derivation is necessary for this technique due to the reason of primary restrictions which remain in the relative non-volatility of drug substances. Gas chromatography has also extended its usability for evaluation of some drugs, i.e., cocaine [43], isotretinoin [44], and used in the resolution of remaining solvents in the betamethasone valerate [45]. It is a very useful criterion for the analysis of pharmaceuticals too. Not long ago, GC by using a variety of detectors has been used for the assessment of the process-related pharmaceutical impurities [46].

9.7.2.3 High-Performance Thin-Layer Chromatography

Undertaking drug analysis seems to be much more comfortable than before due to the development of techniques like HPTLC. It consumes less time with more accuracy due to fast separation, as well as can examine a wide variety of samples. HPTLC is much easier to perform than many other techniques because it is easy to handle and less time required analyzing even the sophisticated and crude sample cleanup. HPTLC is not a time-limited technique and can evaluate the whole chromatogram with a kind of variable. Furthermore, results can be more reliable due to the nonconformist improvement of many samples and quality on each plate. HPTLC is also very useful to determine the number of drugs such as cyproterone, ethinyl estradiol [47], alfuzosin [48], tramadol, and pentazocine.

9.7.2.4 High-Performance Liquid Chromatography

HPLC is recognized as an innovative chromatographic technique mostly used in the chemical and biological structure to get a good understanding of the role of independent molecules by extracting the complex mixture of molecules [49]. HPLC methods were used for the evaluation of heavy drug constituents in 1980 for the first time [50]. HPLC method is particularly useful due to its high specificity and more precision level. The advantageous features of the HPLC method like specificity, precision, and accuracy largely depend on the condition, if widespread system fitness tests are performed before its analysis. Furthermore, the HPLC method is comparatively cost consuming than many other methods. Meanwhile, the HPLC method is still more broadly used among the chromatographic techniques. In liquid

chromatography, more expertise is needed to detect all the components used. For this purpose, a UV detector is mainly used in HPLC due to its capability to detect various wavelengths at one time, which can only be possible by the application of multiple wavelength scanning programs. For better detection of many UV-absorbing components simultaneously, an adequate quantity of UV detector is needed [51].

A photodiode arrangement (PDA) is an edge display of the detached photodiodes on a unified circuit (IC) piece for spectroscopic analysis. PDA is beneficial in spectroscopy, as it has recognized as a border arrangement of separate photodiodes on an integrated circuit (IC) chip. It can sense a wide range of wavelengths simultaneously by placing it at the resemblance strategy of a spectrometer. The application of a different wavelength detector (VWD) sample should be inserted many times with different wavelengths to ensure the detection of all possible peaks. PDA can be helpful in the identification of various compounds at one time that absorbs in its range due to its ability to develop a wavelength range. It can also examine high purity within a peak by going with spectra as well as applicable in pharmaceuticals in the method expansion of iloperidone. The refractive index detector is known to be a detector of choice because it can detect analytes like sugars, alcohols, carbohydrates, polymers, and fatty acids having low or no UV absorption. Better trace detection ability is protected via low noise. This detector is found to be having the least sensitivity as compared to other detectors but still useful for analyte with a high cluster. Lakshmi and Rajesh firstly served the refractive indication detector for voglibose content analysis in pharmaceutical products [52]. For better results of the electrochemical detector, a substance should have the oxidizable or reducible properties, and meanwhile, the chemical reaction occurring at the surface of the electrode results from an electrical output generated by triggering of electron flow. This detector has recently extended its application as being used to examine the quantity of glutathione in human lung adenocarcinoma cells and cancer [53]. Fluorescence detector is known to be the most tactful radar among the LC locater due to its very high responsiveness of about 10–1000 than UV radar for high UV absorbing materials considered advantageous for the measure of precise fluorescent species in samples. The estimation of pharmaceuticals is also considered as the backbone in fluorescence applications. For better consistency, analysis time, sensitivity, and repeatability mostly employ in UV absorbance detection use reversed-phase method. HPLC has been used for numerous drugs being assessed in biological fluids and pharmaceutical formulations, so it has resolved many problems faced in the pharmaceutical industry [54]. On the other hand, HPLC also has some limitations in use due to the high price of columns, solvents, and short-term reproduce ability pertaining to the exclusive nature of column packing.

Liquid chromatography (LC), together with the mass spectrometry (MS), has produced excellent results during the previous decay of the twentieth century. It has got significant importance in the pharmaceutical industry and is a method to choose for analytical support during different phases of quality assurance and quality control. HPLC-MS has also been employed for the evaluation of drugs. The use of HPLC in analyzing the drugs alone can also be applied in combination with the hyphenated technique to determine the impurities of the pharmaceuticals and degradation products [55].

9.7.2.5 Thin-Layer Chromatography

It is known to be an old technique but still got so much importance in various drug product analyses. In thin-layer chromatographic technique, an adsorbent, a solid phase is generally spread on aluminum, plastic, or glass (reliable support) as a thin layer. The effectiveness of chromatographic separation depends on several factors. In this chromatographic technique, the selectivity of adsorbent is significant as it should be highly selective toward the elements being separated as the differences in the elution rate be high. Adsorbent selection is also crucial in the separation of any kind of mixture as they can be a strong or weak adsorbing agent.

Thin-layer chromatography is being used widely for the checking of numerous inorganic and the organic materials due to the following advantages: minimal sample cleanup, huge sample loading range, multiple options of mobile phases, the pliability in sample difference, and less cost [56, 57]. It is also very useful and gained the primary importance for the screening of unidentified materials in bulk drugs as well as shows high degree certainty for the separation of required components of the drug. These properties of the TLC technique, along with its high specificity, extended its use to do quantitative analytical tests following spectrophotometric measurement. Thin-layer chromatography has also been used for the identification of few steroids [58], noscapine [59], pioglitazone [60], and celecoxib [61]. Usually, during the beginning process of drug growth, there is a lack of evidence about the degradation of products and impurities in drug products; TLC plays an essential role during that stage. TLC can also identify many different kinds of degradation products in pharmaceuticals [62, 63].

9.7.3 Spectroscopic Techniques

9.7.3.1 Spectrophotometry

On the bases of natural UV absorption and chemical reactions, spectrophotometric methods are groups of methodologies having a significant part in pharmacopeias. Generally, spectrophotometry can be stated as the measurement (quantitative) of transmission or reflection characteristics of a substance with varying wavelengths. These techniques are considered to be better than many others due to their property of less time and labor consumption, along with a high precision rate. A combination of UV-vis spectrophotometry has played an essential role in the examination of pharmaceutical dosage form during the past few years [64, 65].

Usually, colorimetric methods consisted of the following three parameters:

(i) A catalyze reaction
(ii) Compound formation reaction
(iii) Oxidation-reduction reaction

Colorimetric procedures have been employed permanently for the assessment of large materials, such as drug formulations (corticosteroids), which are usually determined by the blue tetrazolium assay [66]. This technique is also used for the

assessment of cardiac glycosides mentioned in the European Pharmacopeia. For qualitative analysis and assessment, the derivative of spectroscopy that uses the upper or first derivatives of absorbance following wavelength is being employed. Derivatizing the spectral data concept came out in the 1950s, offering many advantages. On the other hand, this technique could not get high consideration, especially due to the difficulty of creating derivative spectra using primary UV-visible spectrophotometers. In the late 1970s, the introduction of microcomputers made it comparatively easy to use mathematical methods for the generation of derivative spectra more rapidly and reproducibly, which increase the use of this technique. The derivative method has extended its uses beyond UV spectrophotometry to infrared, atomic absorption, fluorimetry, and fluorescence spectrometry. This method is not limited in its use but can also help solve problems in the quantitative study of standard spectra. Derivative methods have also shown some disadvantages, such as the discrepancy breaks down the signal-to-noise ratio, so more smoothing is needed in combination with discrepancy [67].

9.7.3.2 Near-Infrared Spectroscopy (NIRS)

NIRS is fast as well as a nondestructive technique that encompasses the testing of multiple constituents of almost any matrix. In the current era, NIRS has obtained a broader appreciation in pharmaceutical manufacturing industries for process monitoring, testing of raw materials, and product quality control. The increased pharmaceutical attention toward NIRS is perhaps due to its main advantages over other analytical procedures, like expectancy of physicochemical parameters of the sample from the single spectrum, and involves easy preparation of sample and likelihood of the separating sample measurement site through the usage of fiber optic probes. The main pharmacopeias have opted for NIR procedures. USP, as well as EP in chapter 2.2.40, mentions the appropriateness of the NIR technique for testing pharmaceutical samples. NIRS, together with multivariate data examination, unlocks numerous stimulating insights in medicinal testing, both quantitatively and qualitatively. Many research papers covering NIR quantitative analysis of active pharmaceutical contents in whole tablets have been published [68, 69]. Additionally, numerous review literatures have been issued, quoting the uses of NIRS in the testing of pharmaceuticals [70]. NIR has been extensively useful for traditional Chinese medicines (TCM) testing for both polar and nonpolar constituents. NIRS for TCM constituents, which are correlated to distinct complexes like saponins, carbohydrates, lipids, essential oils, phenolics, and alkaloids, is used [71]. For quantitative testing of an active ingredient in diverse manufacturing stages of solid dosage forms, the content of the drug is measured at two phases, i.e., after granulation as well as after tablet coating in the pharma industry [72]. NIRS is consuming a portable instrument (microNIR) connected with chemometrics mockups, hierarchical cluster analysis (HCA), and principal component analysis (PCA), and partial least squares (PLS) regression has been applied to determine cocaine as well as to segregate synthetic drugs through functional chemical configuration in 23 medicine samples,19 ecstasy tablets, and 22 seals of designer drugs [73]. NIR process was established, which allows determining olanzapine precisely as

well as accurately in commercial drug products with slight pretreatment of the sample [74].

9.7.3.3 Nuclear Magnetic Resonance Spectroscopy (NMRS)

Arena of NMR-based screening has progressed rapidly after the appearance of the first report in 1996, revealing the application of NMR spectroscopy in order to screen drug molecules [75]. Over the last few years, many advanced methods have been introduced and, also, extensive use of this technique in academic as well as pharmaceutical research [76, 77]. NMR spectroscopy has made its position in analytical testing in order to characterize the composition of the pharmaceutical products, analysis of impurities, and quantification of active ingredients present in biological fluids as well as in pharmaceutical formulations [78–80]. NMR has an essential part in drug discovery development, enabling it to develop as well as evolve its role constantly. NMR is likely to uphold this evolution over the upcoming 10 years with advancements in-cell imaging techniques, automation, speed of structure calculation, as well as the expansion of NMR amenable goals. NMR being adaptable is effective in elucidating high-affinity ligands for biological macromolecules, recognizing small molecules with wide-ranging binding affinity, elucidating ligand-binding sites, as well as it has a major role in structure-based drug designing, and therefore evidencing to be appreciated. Such NMR screening procedures have been built on target observation as well as ligand resonances presenting as detection tool for the identification of weak binding composites and also helping their progression into potent inhibitors (drug-like) for employing in drug discovery as a lead compound. NMR spectroscopy has developed into a significant method in the provision of structure-based drug designing. NMR plays a vital role in the stability analysis of cyclotide cysteine, ethylene-vinyl acetate (EVA) copolymer, β-galactosidase [81]. It is also useful in the determination of drug impurities and also for the quantitative analysis of drugs in biological fluids and pharmaceutical products [75, 78].

9.7.3.4 Phosphorimetry and Fluorimetry

The manufacturers of pharma manufacturing units always show interest in sensitive testing procedures consuming small sample size. Fluorescence spectrometry is a procedure that gives high sensitivity without losing precision as well as specificity. In the recent past, an increased amount of literature covering fluorimetry [82, 83] as well as phosphorimetry [84, 85] usage during quantitative measurements of several active pharmaceutical contents in drug products as well as in biological solutions, have been seen.

9.7.4 Electrochemical Methods

Over the last few years, electrochemical procedures are being used rapidly in the testing of pharmaceuticals. The attention toward electrochemical techniques can be accredited to have more sophistication in instrumentation and easily

comprehensible. Furthermore, numerous electroanalytical techniques are being presented for the pharmaceutical product's quantitative analysis. In order to quantify desipramine-imipramine, trimipramine, titanium, ambulate XAD-2, and dioxide nanoparticles, altered glossy carbon paste was established [86]. Adsorptive stripping differential pulse voltammetry, chronocoulometry, electrochemical impedance spectroscopy, and cyclic voltammetry were used to analyze the electrochemical response of these drugs. Capsaicin adapted carbon nanotube-altered basal plane pyrolytic graphite electrode, and p-chloranil-altered carbon paste electrodes have been introduced for quantification of lidocaine and benzocaine [87]. In order to determine norepinephrine, levodopa, adrenaline, and dopamine, copper (II) complex, as well as silver nanoparticle-altered glassy carbon paste electrode, was used [88]. The electrochemical response of such medications was considered employing adsorptive stripping square wave voltammetry, electrochemical impedance spectroscopy, and cyclic voltammetry and chronocoulometry techniques [89]. Electrochemical response by clioquinol having widespread clinical applications was analyzed by square wave voltammetry and cyclic differential pulse voltammetry at a wide range of pH employing a glassy carbon electrode. The adsorptive stripping differential pulse voltammetry technique was established for the estimation of venlafaxine as well as desvenlafaxine using a Nafion-carbon nanotube compound glossy carbon electrode. Carbon nanotube paste electrode altered at a precise place by Triton X100 was established for separation as well as the concurrent identification of caffeine, aspirin, and acetaminophen. In order to quantify the sub-nanomolar concentration of bismuth, an electrochemical procedure based on potentiometric stripping testing using cryptand as well as carbon nanotube-altered paste electrode has been proposed. An innovative technique, capillary electrophoresis (CE) along with a perimetric detector, has been developed for quick as well as the practical determination of benserazide and levodopa along with its impurity in co-beneldopa pharmaceutical preparations [90]. Electrical methods have been used for quantitative measurement of desipramine imipramine, trimipramine, lidocaine, and benzocaine, levodopa, norepinephrine, epinephrine, and dopamine as well as for effective determination of benserazide and levodopa and its contamination (R, S)-2-amino-3-hydroxy propanohydrazide in co-beneldopa therapeutic preparations [87–90].

9.7.5 Kinetic Method of Analysis (KMA)

This procedure of testing has been emerging since the 1950s; additionally, until now, this is winning the main revival inactivity. The monotonous attention in these procedures can be attributed to the developments made in principles, in comprehending the chemical as well as instrumentation, in investigational data approaches, and in the testing application and automated instrumentation. It is evident from the literature that the kinetic method of analysis is somewhat general, having many benefits over the conventional equilibrium method. Fundamentally, kinetic procedures involve a determination of amount changes (noticed by

alterations in signals) in a reactant with time after mixing of reagents and samples mechanically or manually [91].

It is confirmed from the literature that initial rate and fixed-time methods have been applied frequently for the determination of drugs in dosage forms. Automatic approaches for KMA are usually based on open systems. Stopped flow systems, as well as the continuous addition of reagent (CAR) techniques, are among the standard techniques [92]. Many drugs have been measured with the help of the CAR technique, having photometric as well as the fluorometric detection system. In order to make analytical reactions quicker, catalysts can be used as they are feasible with both equilibrium estimations and reaction rate. Now the application of micellar media in the KMA is appreciated in order to augment the rate of reaction via micellar catalysis. Besides, it may enhance the selectivity as well as sensitivity, which in turn decreases the time for analysis of the desired active ingredient.

Multicomponent determinations by a kinetic method of analysis, most often stated as differential rate procedures, are getting full attention in the pharmaceutical exploration area. In order to deal with the component's overlapping spectra in binary mixtures, two novel methods, i.e., H-point standard addition method as well as kinetic wavelength pair method, have been recommended [48].

9.7.6 Electrophoretic Procedures

Capillary electrophoresis is another significant instrument vital for the testing of pharmaceutical formulations. CE is a comparatively innovative procedure having the basis of segregation of analytes carrying charge by little capillary underneath the influence of the electric field. In a procedure, solute particles are considered as peaks as these analytes pass through the detector, while the specific peak area is directly proportional to their amounts that permit quantification. Electrophoretic procedures are also used for the testing of biopolymer as well as inorganic ions. Capillary electrophoresis testing is usually more efficient, takes less time for analysis, lesser up to nanoliter injection volumes, involves only a small quantity, and, in many instances, happens in aqueous mediums [93]. These four features of this technique have confirmed to be valuable to many pharmaceutical uses. Numerous reports have seemed on the usage of this procedure in the testing of the drugs. Many approaches of capillary electrophoresis such as micellar electrokinetic chromatography electrophoresis, capillary gel, isoelectric focusing, affinity capillary electrophoresis, isotachophoresis, and capillary zone electrophoresis were established and used for pharmaceutical purity analysis as well as in bio testing of pharmaceuticals [94, 95].

9.7.7 Flow Injection Analysis/Sequential Injection Analysis (FIA and SIA)

In the second half of the 20th era, laboratory automation was presented. Ruzicka and Hansen in Denmark, as well as Steward in the USA, made the FIA method for

automation of the chemical process [96, 97]. This procedure emerged to alter the beginning of automation in the chemical testing by allowing instrumental quantification in the absenteeism of chemical as well as physical equilibrium. FIA method's principle is the inoculation of a fluid sample into a non-segmented, flowing, and continuous carrier stream of an appropriate fluid. The inoculated sample makes a zone moving to a detector, which analyses fluctuations in the electrode potential, absorbance, and additional physical restriction occurring bypassing analyte into the flow cell [98, 99]. Flow injection testing steps can be seen in Fig. 9.1.

After the extensive use of computers in the lab, the second-generation flow testing was presented by [100], who named it SIA. Although it is the same as flow injection analysis, being a non-segmented continuous flow process founded on the similar basic concept of controlled dispersion as well as reproducible operation of FIA discernment, its working method includes the basis of programmable flow theory.

FIA method has hired a noteworthy input for the automation development in pharma testing, and its benefits are fully recognized in a specialized monograph [101] and numerous review articles [102]. The SIA technique has made the scientific community to take an interest in automation in the pharmaceutical testing domain. Several articles are published regarding pharmaceutical testing, comprising two review articles using sequential injection testing techniques to extensive diversity of matrices, like solid matrices, i.e., liquids (solution, suspensions, emulsions) and pastes (creams, ointments), as well as encompassing several entities having different healing properties. By profiting from the benefits in the elevated sampling rates as well as the economy of reagents, the major uses are devoted to the quantification of API for quality control in medicinal preparations [103].

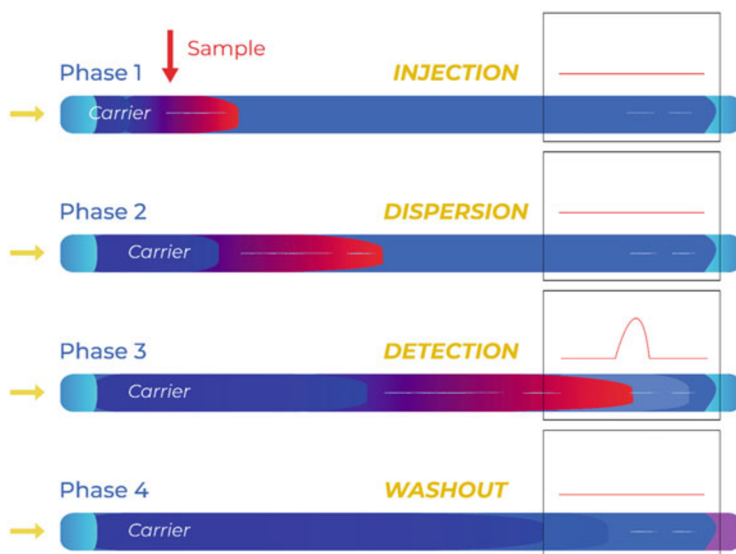

Fig. 9.1 Different steps of flow injection analysis. (Adapted from Refs. [98, 99])

9.7.8 Hyphenated Techniques

Hyphenated techniques develop from the linkage of separation technique with online separation technique. Previous 20 years observed a notable development in the hyphenated methods and their use in medicinal testing. A wide range of hyphenated techniques like GC-MS, CE-ICP-MS, LC-NMR, CE-MS, and LC-MS [104] has been used for testing of pharmaceutical products [105]. The measurement of medicinal entities in biological fluids is a significant phase in drug discovery as well as drug development. High-performance liquid chromatography with different types of detectors like PDA mass spectrometry and fluorescence has become the method of choice for testing pharmaceuticals products. A review of high-performance liquid chromatography with PDA or MS/MS detector is reported for testing meloxicam in biological samples as well as medicinal products. For qualitative as well as quantitative testing of metabolites of *Zuojinwan Rhizome* and *copies* formulation; after oral intake in the rat urine, a liquid chromatography along electrospray ionization mass spectrometry has been established. The same testing method was used for the instantaneous quantification of acetylsalicylic acid as well as L-ascorbic acid in aspirin C effervescent tablets [106]. C_{18} column is used for the separation of urine samples with the help of a mobile phase containing water and acetonitrile having formic acid (0.1%) in the ratio of 70:30. Abuse of recreational drugs is a rising problem, and innovative materials are commonly noticed in forensic and clinical samples. Diphenyl-2-pyrrolidinemethanol is one of such items. So, this and its biologically degraded products in rat urine have been identified with the help of GC-MS and LCMS. Trials were made to detect the existence of humanly used medicinal entities in the aquatic areas of Malaysia territory. Required aquatic testing samples are assembled from diverse locations along Langat River as well as effluents from 05 sewerage treatment plants after solid-phase extraction and tested with the help of LC having a tandem mass spectrometry detection system [107]. This analytical investigation has proved the existence of glibenclamide, salicylic acid, and mefenamic acid in all the river water samples. Drug-drug interaction of clopidogrel, as well as rabeprazole in a healthy Chinese population, has been investigated. After oral intake, the amount of rabeprazole, as well as clopidogrel blood, has been analyzed by using liquid chromatography linked with mass spectrophotometry. A new liquid chromatography linked with the mass spectrophotometry-MS technique was established for the identification of bacterial isolates' carbapenemase response. Liquid chromatography-tandem mass spectrometry was used to evaluate the distinctive pharmacokinetic parameters of the individual analyte like caffeine, midazolam, warfarin, omeprazole, and metoprolol [108].

9.7.9 Thermal Analytical Techniques

The existing arena of thermal exploration is both diverse and dynamic. Theoretically, entire thermal analytical methods evaluate the alterations of a particular attribute of material with a varying temperature that leads scientists to have

Table 9.3 Thermal analytical methods and measured properties

Sr.#	Analytical technique	Measured property
1	Dynamic mechanical analysis (DMA)	Deformation
2	Evolved gas analysis	Gaseous decomposition
3	Dielectric thermal analysis (DEA)	Deformation
4	Thermogravimetric analysis (TGA)	Mass
5	Differential scanning calorimetry (DSC)	Enthalpy
6	Differential thermal analysis (DTA)	Temperature difference
7	Thermo-optical analysis (TOA)	Optical properties

information regarding macroscopic concepts of matter like irreversible kinetics and thermodynamics as well as equilibrium. Merely a few practices are commonly used in the pharmaceutical sciences. These involve differential scanning calorimetry (DSC, differential thermal analysis (DTA), dynamic mechanical analysis (DMA), and thermogravimetric analysis (TGA). Table 9.3 describes the properties measured by several thermal analytical techniques [109, 110].

9.7.9.1 Differential Scanning Calorimetry (DSC)

The conception of DSC was formerly originated from earlier DTA apparatuses. The primary difference between DSC and DTA is that theoretically, DSC measures enthalpy change, while DTA determines a difference in temperature of reference as well as a sample [111, 112]. The International Confederation for Thermal Analysis and Calorimetry (ICTAC) explains that DSC is an analytical technique for measuring heat flow rate change between reference material the sample. Commercially two major categories of DSC apparatuses exist, i.e., power compensation DSC (pc-DSC) and heat flux DSC (hf-DSC), originally denoted to as quantitative DTA. At this time, there is another significant difference that no common method is available for presenting DSC data. Based on the apparatus being employed, the default settings could show exothermic measures in a downward direction while endothermic measures in an upward direction [113].

9.7.9.2 Thermogravimetric Analysis (TGA)

TGA is a procedure in which a difference in mass is detected and determines the physicochemical processes that happen upon heating the test sample. Simplified TGA apparatuses design consists of an accurate analytical balance attached with a sample pan, suspended within a heater, which in turn controlled by computer. TGA results like DSC may differ prominently based on test material as well as experimental [114, 115]. Thermogravimetric analysis is precious for studying numerous kinetic procedures of solids/liquids as far as the methods encompass mass loss. This can be attained by the use of accurate balances, also including accurate control of rates of heating and cooling and atmospheric circumstances. Additional common uses in pharmaceutical sciences involve hydrate characterization comprising an evaluation of decomposition, sublimation temperatures, or vaporization and desolvation procedure. Though most thermal analytical techniques involve the

characterization of samples as solids, semi-solids, or liquids, common applications encompass:

- Characterization of the physicochemical attributes of crystalline solids.
- Identification as well as detection of polymorphism.
- Due to increased utilization of solid dispersions as well as other polymeric formulations, thermal analytical techniques are employed more frequently for development and characterization.
- To study the effects of lyophilization as well as to develop optimal lyophilization dosage forms and cycles [116].

9.8 Conclusion

The key purpose of the pharmaceuticals is to assist the human so that they can avoid possible ailment or can prevent the disease. For achieving this anticipated purpose of medicines, they must be free from all types of impurities and other factors that may cause any harm to humans. In this chapter, our main target was to discuss drug stability and which type of different analytical techniques could be utilized for the assessment of stability and quality of drug substance. Various analytical techniques for the assay of drugs are discussed with detailed instrumentation involved. This chapter also highlighted the applications of these analytical techniques and their development from the old trimetric method to the latest hyphenated technique.

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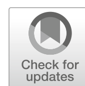

Stability of Pharmaceutical Products

10

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Abstract

This chapter explains the concept of stability of pharmaceutical products as the extent to which they retain those properties and characteristics within the specified limits, throughout their period of storage and use, possessed at the time of their packaging. The terms like shelf-life and expiry are well defined here. The importance of stability of drug products describes the consequences of instabilities occurring in drug products. Types of stability mainly physical, chemical, microbiological, therapeutic, and toxicological stability have also been discussed concisely. Factors such as moisture, excipients, temperature, pH, oxygen, and light affect the efficacy, quality, and properties of drugs, which makes the drug either a toxic compound or a substance that has no or very little therapeutic activity. Instabilities among different pharmaceutical drug products and substances are explained along with the stability studies to evaluate their degradation behavior upon exposure to various parameters. Such parameters can be controlled according to the stability study type. The testing methods described in this chapter are real-time stability testing, accelerated stability testing, retained sample testing, and cyclic temperature stress testing along with their advantages and disadvantages. The standards for environmental conditions to be provided during such studies are set according to WHO and ICH guidelines. These studies play an important role during drug development stages and are considered as fundamental processes to be carried out for the approval and

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Springer Nature Singapore Pte Ltd. 2020
M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_10

acceptance of pharmaceutical drug products in the market. These are responsible for the evaluation and prediction of shelf-life of the pharmaceutical product and the determination of appropriate storage conditions as well.

Keywords

Stability · Shelf-life · Real-time stability testing · Accelerated stability testing · Retained sample testing · Cyclic temperature stress testing

10.1 Introduction

The ability of pharmaceutical product that is present in particular closure system to conform its physical, chemical, toxicological, microbiological, informational, and protective specifications is referred to as the stability of pharmaceutical dosage form and/or product. This can also be defined as the degree to which the formulation maintains its quality in every aspect from the time of packing to storage period or shelf-life [1]. Shelf-life can be explained as when the concentration of a drug or a substance decreases to 90% from its initial concentration. This terminology is used to indicate the product stability; the commonly used term in place of shelf-life is the expiry date. There are some environmental factors that affect the expiry of drug products particularly temperature, light, radiations, humidity, etc. Physical and chemical parameters are also involved that influence the stability of pharmaceutical drugs [2]. From the process of manufacturing to the final packing, stability of pharmaceutical products is maintained to sustain its identity, efficacy, purity, potency, and quality. Therefore, stability tests are put through during different stages of development of the product. This type of testing procedure is relatively expensive and time-consuming and requires many technical skills [1].

10.2 Significance of Studying the Stability of Drug Products

I. Low concentration of drugs in pharmaceutical dosage form due to the instability of the product can bring about the undermedication than the recommended dose.
II. Toxic compounds with deleterious effects can be produced as a result of decomposition. For example, Fanconi syndrome might be a result of epianhydrotetracycline toxicity which is the degraded product of tetracycline [3].
III. While transporting the product to different places, the physical characteristics of the drug can be reduced and will be distasteful for patients.
IV. Principles of kinetics are applied for the prediction of certain instabilities of drugs [4].

10.3 Factors Affecting the Stability of Pharmaceutical Products

Factors that are majorly involved in the instability of pharmaceutical products are as follows:

10.3.1 Moisture

The presence of excess amount of unbound water in the dosage form results in the dissolution of water-soluble active drug substance. Therefore, moisture leads to chemical and physical instability [5].

10.3.2 Excipients

All pharmaceutical drug products have different compatibility status, i.e., every drug is not compatible with any type of excipients as some of them contain relatively high moisture content. So this will cause drug-excipient interaction and instability of drugs as well [6].

10.3.3 Temperature

Chemical degradation reactions such as hydrolysis take place in consequence of increased temperature. The resultant degraded product could have injurious effects on the health of patients.

10.3.4 pH

The changes in the value of pH in the dosage form cause epimerization reactions. To avoid such reactions, buffering agents are introduced in drug products.

10.3.5 Oxygen

The chances of oxidation reactions rise in drugs due to the presence of oxygen. This can be prevented if nitrogen or carbon dioxide is purged into the closure system containing active pharmaceutical ingredients.

10.3.6 Light

Light is one of the important contributing factors that affect the stability of photolabile or photosensitive drugs. As photolytic decomposition of such drugs

increases upon exposure to light, they are preferably stored in dark or amber glass containers [7].

10.4 Types of Stability of Pharmaceutical Products

Stability of drugs is classified into five main classes, as shown in Table 10.1.

10.5 Classification of Pharmaceutical Products with Respect to Stability Studies

The degradation of pharmaceutical drug products and substances has been investigated through different stability-indicating methods, to calculate the amount of drug left behind after the degradation process with respect to time. These methods measure the concentration of active ingredient precisely, despite the presence of other degraded products, impurities, and excipients [8]. Some of the pharmaceutical products, such as oral solutions, suspensions, parenteral, tablets/capsules, and emulsions (Table 10.2), are typically susceptible to get degraded or decomposed.

Table 10.1 Classes of stability of drugs

Types of stability of pharmaceutical products	
Physical stability	Physical stability refers to the maintenance of the physical properties of drugs that include its appearance, content uniformity, palatability, dissolution, and suspendability. Physical instability reactions are:
	Precipitation
	Sorption
	Leaching
Chemical stability	Chemical stability refers to the preservation of the chemical properties of the active ingredients and labeled potency. Chemical instability reactions are:
	Hydrolysis
	Oxidation-reduction
	Photolysis
	Racemization
	Epimerization
Microbiological stability	Microbiological stability refers to the maintenance of sterility of the drug product and prevention of the drug from the contaminants mainly microorganisms
	A high level of moisture content leads to microbiological instability due to the growth of microbes in it causing the degradation of the product
Therapeutic stability	Therapeutic stability refers to keeping the efficacy and effectiveness of the drug throughout its time of storage
Toxicological stability	Toxicological stability refers to the controlling of toxic metabolite production as a result of the degradation of the drug product

Table 10.2 Instabilities in pharmaceutical products

Pharmaceutical products	Stability problems
Oral solutions	Loss of flavor
	Precipitation
	Discoloration
Suspensions	Crystal growth
	Loss of drug content uniformity
	Decrease in shelf-life
Parenteral	Presence of microbial growth
	Change in odor
	Change in color
Tablets/capsules	Discoloration
	Change in appearance
Emulsions	Cracking
	Phase inversion
	Presence of microbial growth
	Change in odor

Table 10.3 Storage conditions for real-time stability studies

Parameters	ICH guidelines	WHO guidelines
Temperature	25 °C ± 5	25 °C ± 5
Relative humidity	60%	60%
Duration	12 months	6 months

These instabilities may occur due to photolysis [9], presence of moisture or microbial content, and most importantly changes in pH and temperature.

10.6 Stability Evaluation Studies

In different stages of drug product development, it is necessary to test the drugs and evaluate their stability at various storage conditions. At initial phases, drugs are evaluated at a comparatively extreme condition that is referred to as accelerated stability testing. For the evaluation of the pharmaceutical product's shelf-life and expiry, relatively less severe and long period shelf storage conditions are endorsed [10]. Relying upon the situation, the following testing procedures are executed:

10.6.1 Real-Time Stability Testing

In this type of evaluation, the time period for studying the stability behavior of drug is sufficiently longer or equal to 6 months so that notable degradation of product occurs at specified storage conditions [11]. In other words, the storage conditions are not artificially induced. They are actually tested under the natural climate state as shown in Table 10.3.

10.6.2 Accelerated Stability Testing

In this stability testing method, the stability of drug products is evaluated under accelerated storage conditions, mainly temperature. Such a pressured environment generally includes acceleration temperature, moisture, light, agitation, pH, gravity, packaging, and manufacturing method. The objective behind this study is to get an early prediction of the shelf-life of the drug products and lessen the duration of testing procedure, i.e., it can be accomplished within just a few days or weeks [12]. This test can be performed by using the most common approach, that is, by Arrhenius equation. Arrhenius relationship expresses the relationship time and stability of a product stored under constant conditions. It is dependent on the rate constant and order of the reaction. The logarithm of the rate of reaction is a linear function of reciprocal of absolute temperature [13]. It can be expressed as:

$$\log\left(k_2/k_1\right) = \left(-E_a/2.303R\right)\left[\left(1/T_2 - T_1\right)\right]$$

In the above mathematical equation, k_2 and k_1 represent the rate constants at temperature T_2 and T_1 in kelvins, respectively. *Ea* denotes the activation energy and R is the gas constant. This Arrhenius relationship helps in predicting the rate of degradation at elevated temperatures or any other preferred temperature. In this way, the shelf-life of the pharmaceutical product can be estimated. The rate of reaction at different temperatures is approximated through rate constants. Activation energy E_a is the independent variable, whose value should be higher for the occurrence of degradation reaction.

10.6.3 Retained Sample Stability Testing

This type of evaluation is carried out for almost every marketed product for which stability data are required. In retained sample stability testing, the samples are selected for at least one batch a year, whose stability for retained storage is being examined. In case if the quantity exceeds 50 marketed batches, then stability samples preferably from two batches are taken. When the drug sample is to be launched for the first time in the market, then it is recommended to draw stability samples from every batch, and it can be reduced to only 2–5% of marketed batches at a later stage. The time intervals are already determined in this study, assuming if a drug product has a shelf-life of 5 years, it is conventional to test samples at 3, 6, 9, 12, 18, 24, 36, 48, and 60 months [14].

10.6.4 Cyclic Temperature Stress Testing

It is not a routine testing procedure for marketed products. This is formulated based on the knowledge of the drug product, thus resembling the environmental conditions at the market storage place. The duration of the cycle is primarily 24 h as the diurnal

rhythm on earth is 24 h. On the basis of product type, the minimum and maximum values of temperature are suggested, and chemical and physical degradation factors affecting the drug characteristics are also considered. The acceptable number of cycles should be equal to 20 in this study.

10.7 Advantages of Stability Studies of Pharmaceutical Products

I. Real-time stability testing enables us to study the shelf-life of a drug under different geographical climate changes.
II. The approximated shelf-life and properties of drugs in accelerated stability studies can be evaluated in a shorter period [15].
III. In accelerated stability studies, the rate of degradation reactions either chemical or physical of pharmaceutical products is sped up. The shelf-life and labeling instructions can be anticipated using these stability testing methods.

10.8 Disadvantages

I. Real-time, retained sample, and cyclic stability temperature stress studies consume so much time.
II. Arrhenius equation in accelerated stability studies considers only the temperature, whereas the other factors involved in instability, like relative humidity and presence of oxygen, are not contemplated.

10.9 Conclusion

Stability of a pharmaceutical drug product is the most essential factor that should be maintained, as the efficacy and therapeutic activity of an active ingredient in the drug product can be lost due to some environmental factors like temperature, oxygen, pH, moisture, and light. Those dosage forms that contain water content are more prone to microbial contamination that could result in infectious diseases, and conditions could be severe in a case when parenteral carries pyrogens. Therefore, all dosage forms must be kept under controlled storage conditions. During the development of new drug products, proper storage conditions for that product can be evaluated through stability studies, and also suitable instructions for labeling of the finished product are estimated. The benefit of this type of testing is that the degraded product of the active ingredient formed as a result of the chemical or physical reaction is identified; moreover, its effect is also determined.

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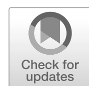

Role of Kinetic Models in Drug Stability 11

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Abstract

Kinetic models are studied to determine their role in stability and release kinetics of drugs. The mathematical models that have been widely used are zero-order kinetic model, first-order kinetic model, Higuchi model, Korsmeyer-Peppas model, and Hixson-Crowell model. Although they have vast applications, there are some factors that may effect on mechanical, physical, pH, relative humidity, and presence of solvents. Several types of drugs are being fitted in zero-order and first-order kinetic models, while a polymeric matrix system containing drugs is preferably fitted in Higuchi model, Korsmeyer-Peppas model, and Hixson-Crowell model. In this chapter, we have briefly discussed in detail the role of these aforementioned kinetic models in the stability of various types of drugs and their dosage forms.

Keywords

Zero-order kinetic model · First-order kinetic model · Higuchi model · Korsmeyer-Peppas model · Hixson-Crowell model

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_11

11.1 Introduction

Chemical kinetics deals with the changes in chemical properties of drug and any substance over time and is mainly related to the rate of changes. It is interpreted as rate of reaction in which concentration **c** is changed with respect to time *t* (indicated mathematically as dc/dt). Factors that are mainly involved in the rate of reaction are concentration of reactants, temperature, catalysts, and various environmental conditions [1]. Information and description of different types of reactions including their mechanisms can be shown through kinetic models. Various types of reactions particularly the Michaelis-Menten reaction, mass action, and some other parameters like the catalytic constant ($k$cat), maximal rate of reaction (Vmax), and Michaelis-Menten constant (km) can be described by kinetic models [2]. These mathematical models express the relationship between the variables and parameters, which helps the researcher to demonstrate the ideas about the process under research [3].

Stability of drug plays an important role in drug efficacy and its activity. Study of drug stability using different kinetic models has been developed in order to explain the course of reaction mathematically. For this purpose, stability studies are conducted that ensure that the drugs lie within the range of acceptance criteria and are unaffected by environmental or non-environmental factors that may involve in causing instability of drugs. Factors primarily involved in instability of drug are taken into account as they can cause loss in quality and efficiency as well as increase and/or decrease in concentration of drug in dosage form and also lead to decomposition and degradation of it. These types of instabilities may occur during shelf-life and transportation of drugs. Chiefly, three types of drug stabilities are considered to be maintained during such conditions, i.e., physical, chemical, and microbiological [4].

11.2 Types of Stability of Drugs

Three types of drug stabilities are considered to be maintained during such conditions, i.e., physical, chemical, and microbiological. Their detailed description has been discussed in the following subsections:

11.2.1 Physical Stability

Physical stability includes stability of physical properties of the drug in terms of its organoleptic characteristics, change in its appearance, size and shape of particle, content uniformity, and pH. These physical alterations are reasonably due to vibration, abrasion, and temperature variation. The abovementioned physical characteristics are necessary to be maintained throughout stages of drug development and shelf-life [5, 6].

11.2.2 Chemical Stability

The changes occurring in chemical constitution of drug formulation are referred to as chemical instabilities. Chemical reactions going down in pharmaceutical product are mainly hydrolysis, oxidation, photolysis, solvolysis, oxidation, reduction, and racemization. Such variations lead to loss of therapeutic activity and efficacy of active pharmaceutical ingredient (API) and also generation of toxic products. Liquid dosage forms are more prone to chemical degradation than solid dosage forms [5].

11.2.3 Microbiological Stability

Microbiological stability of drugs includes the stability of drug formulation in respect to its sterility, i.e., free from any microbial contamination (bacteria or fungi). The safety of drug product could be compromised in case of any microbial growth especially in semi-solid drug products and liquid dosage forms due to the presence of water or moisture. Microbiological instability can be controlled in these formulations by adding preservatives or antimicrobial agents [5]. To identify about the type and cause of instability, forced degradation studies are executed in order to understand the stability behavior of an active pharmaceutical ingredient. With the help of these studies, information related to degradation pathways of drug products can be obtained [7]. Types of these studies are shown in Table 11.1.

11.3 Kinetic Models

Kinetic models are employed to study the abovementioned stabilities in pharmaceutical drug products. After gathering data through various experimental procedures, an appropriate model is appointed in order to evaluate their stability behavior [8]. These models then manifest the results of data being fitted in these models, by the help of their respective equations. The most commonly used kinetic models in drug stability and drug release are:

Table 11.1 Types of stability studies

Type of study	Storage conditions	Time duration
Long term	25 °C ± 2 °C and 60% RH ± 5% RH or 30 °C ± 2 °C and 65% RH ± 5% RH	12 months
Intermediate	30 °C ± 2 °C and 65% RH ± 5% RH	6 months
Accelerated	40 °C ± 2 °C and 75% RH ± 5% RH	6 months

11.3.1 Zero-Order Kinetic Model

In zero-order kinetic model, the rate of reaction is independent of the concentrations of reactants; thus, the rate will be represented as:

$$\text{Rate} = \frac{\mathrm{d}[A]}{\mathrm{d}t} = k[A] = k$$

where A represents the reactant and k is a zero-order rate constant.

11.3.2 Role

- Mostly osmotic pump delivery system and transdermal delivery system and matrix system for drugs with low solubility follow zero-order kinetic models [9].
- The stability study of powder for injection and reconstituted sample of third-generation cephalosporin, ceftazidime, was conducted. Stress conditions like temperature and ultraviolet and visible radiation were applied to study their kinetics of degradation. For reconstituted sample and powder for injection, zero-order and second-order kinetic models were utilized to explain the degradation phenomena of ceftazidime [10].
- Thermal stability of drug that is used in the treatment of diabetes mellitus, i.e., metformin hydrochloride (1,1-dimethylbiguanide hydrochloride), was studied by using kinetic models. This drug was exposed to temperatures 30, 40, 50, and 70 °C in aqueous medium. The gathered data was treated by Arrhenius, zero-order, and first-order kinetics. It was evaluated that thermal degradation of metformin followed zero-order kinetics and decomposed up to 10% in 208 h [11].
- The level of scopolamine in plasma after the application of transdermal patch on the skin was anticipated through theoretical model of transdermal drug delivery system. The drug released from this system followed zero-order kinetics. The rate constant of zero-order kinetics in this type of system is related to the diffusional characteristics of scopolamine [12].
- An erodible device which maintains the constant surface area with respect to time was used in order to measure the release kinetics of amoxicillin. The prediction made by using this device elicited that amoxicillin follows zero-order kinetics apparently from the planar surface of tablet [13].

11.3.3 First-Order Kinetic Model

This model is widely used in the stability studies of pharmaceutical products. In this first-order kinetic model, the rate of reaction is directly proportional to the concentration of the reactants:

$$\text{Rate} = \frac{\mathrm{d}C}{\mathrm{d}t} = -Kt$$

The time at which the concentration of reactants decreases to 50% from its original concentration is referred to as the half-life denoted as $t_{1/2}$. The half-life of first-order kinetics can be determined by these formulas:

$$t_{1/2} = \frac{\ln 2}{k}$$

$$t_{1/2} = \frac{0.693}{k}$$

11.3.4 Role

- The porous matrices in dosage forms that contain water-soluble drugs follow first-order kinetics, as their release is proportional to the concentration of drug [14].
- Degradation profiles of drugs like metronidazole, tetracycline, and famotidine can be determined with the aid of these kinetic models. This triplicate therapy is used in the treatment of *Helicobacter pylori*-associated peptic ulcer. The stability and compatibility of these drugs in different states were studied which were exposed to mild to extreme conditions. It was concluded that the degradation profiles of these drugs follow pseudo-first-order kinetics [15].

11.3.5 Higuchi Model

This model was proposed in 1963 by Higuchi to explain the release kinetics of drugs from the matrix system. Model expression is given by the equation:

$$Q = A[D(2C - C_S)C_S \quad t]^{1/2}$$

where "Q" represents the quantity of drug released in time t per unit area A, "C" represents the drug initial concentration, Cs is solubility of drug in the media, and diffusivity of molecules (diffusion coefficient) is denoted by "D" in the matrix. The simplified form of this model is represented as:

$$Q = K_H\sqrt{t}$$

The above equation demonstrates the direct relationship between cumulative quantity of drug released and square root of time. The proportionality constant or Higuchi constant, i.e., K_H, can be obtained by the slope of graph that has some physically realistic and specific meaning.

11.3.6 Role

- The equation derived by Professor Takeru Higuchi facilitates quantification of release of drug from different dosage forms, for example, release of finely dispersed drug from thin ointment film under sink conditions.
- Kinetic models are used in the in vitro stability studies of different pharmaceutical dosage forms. For example, aspirin-magaldrate double-layer tablets were compared with marketed Ascriptin® and Aspro®. They were stored at different temperatures for specified period of time. The best fitted model to study the stability was first-order kinetic model when storage temperature was 70 °C and Higuchi model when storage temperature was 50 °C and 60 °C with the time period of 50 days. The result of this study showed that the presence of alkaline moieties in aspirin-magaldrate double-layer tablets reduced the shelf-life and increased the rate of decomposition of aspirin [16].

11.3.7 Korsmeyer-Peppas Model

Korsmeyer et al. and Peppas explained the phenomena of release of drug from polymeric system. They also formulated an equation for the analysis of Fickian and non-Fickian release of drugs through polymeric delivery system that may be either swellable or non-swellable matrix. The mechanism of release of drug can be found after fitting first 60% drug release data in Korsmeyer-Peppas model. The following equation represents Korsmeyer-Peppas model:

$$Mt/M_{\alpha} = kt^{n}$$

The fraction of drug released at time t is designated as Mt/M_{α}, where k is the release rate constant and n is the release exponent [17]. The value of n characterizes the release mechanism of drug as shown in Table 11.2.

Table 11.2 Drug release mechanism according to Korsmeyer-Peppas model

Mechanism of drug transport	Release exponent (n)	Mechanism of drug release	Rate as f(t)
Quasi-Fickian diffusion	$n < 0.5$	Non swellable matrix diffusion	t^{n}
Fickian diffusion	0.5		$t^{0.5}$
Anomalous (non-Fickian transport)	$0.5 < n < 1.0$	For both diffusion and relaxation (erosion)	t^{n-1}
Case II transport	1.0	Zero-order release	Independent of time
Super case II transport	Higher than 1.0	Relaxation/erosion	t^{n-1}

11.3.8 Role

- Korsmeyer-Peppas and Higuchi models are the best kinetic models for the evaluation of stability of advanced drug delivery systems, as these models not only describe the in vitro release of drug from the matrix system but also explain the stability behavior. The compatibility between drug-polymer, content of drug, and encapsulation efficiency was studied in intravaginal mucoadhesive microspheres of tenofovir disoproxil fumarate (TDF). Accelerated stability study was followed to conduct that research [18].

11.3.9 Hixson-Crowell Model

Hixson-Crowell cube root model is employed for those systems in which the surface area and diameter of the drug matrix change with time. Hixson and Crowell in 1931 discovered that regular area of a group of particles is proportional to the cube root of its volume. The relationship established by Hixson-Crowell is described by the following equation:

$$W_0^{1/3} - W_t^{1/3} = K_{\mathrm{HC}^t}$$

The initial amount of drug at time 0 is represented as "W_0," the amount of drug remaining in the pharmaceutical dosage form at time "t" is represented as "W_t," and Hixson-Crowell constant is denoted as "K_{HC}" which describes the surface-volume relationship [19].

11.3.10 Role

- Hixson-Crowell model was applied to study the efficient release of an anti-schizophrenic drug haloperidol by using vehicle cysteamine hydrochloride protected carbon dots. Under standardized conditions, haloperidol followed the Hixson-Crowell model, i.e., constant release of drug was attained for more than 40 h [20].
- The accelerated stability was conducted on regioselective floating tablets of atenolol and lovastatin, and their degradation profiles were obtained as well as release kinetics. It was evaluated that atenolol followed mixed pattern of kinetic models, among which Hixson-Crowell was present [21].
- A study was carried out to understand the role of various polymers on drug release kinetics of losartan potassium oral controlled release tablets. In that study, through Hixson-Crowell model, a good correlation was obtained. This showed

that the release of drug was affected by the change in surface area of the solid dosage form [22].

11.3.11 Merits and Demerits of Kinetic Models

We have described the merits and demerits of the aforementioned kinetic models in Table 11.3.

Table 11.3 Merits and demerits of kinetic models

Kinetic model	Merits	Demerits
Zero-order kinetic model	It is used to explain dissolution of drugs in many different types of pharmaceutical modified release dosage forms	This model cannot be utilized for all types of pharmaceutical dosage forms
	It helps to describe the release kinetics of drugs in transdermal systems, as well as matrix tablets with low soluble drugs, coated forms, osmotic system	
First-order kinetic model	This model describes the dissolution of drugs which are water soluble present in porous matrices	First-order kinetic model is not always applicable to controlled drug delivery system
Higuchi model	Higuchi model is very easy to use in spite of the complex mass transport process	Due to the occurrence of many advanced kinetic models, the Higuchi equation could be confused with other equations while implementation or study of release kinetics of drugs [23]
	This type of model helps in understanding the release of drug mechanism from controlled drug delivery systems, e.g., transdermal patches, matrix tablets, etc. where water-soluble drug is being incorporated	
Korsmeyer-Peppas model	This type of model aids in linearization of data obtained from the release profile of several formulations of microcapsules or microspheres	The researcher while using this model could get confused when explaining the release pattern of drugs
	This model describes the Fickian and non-Fickian release pattern of drugs	
Hixson-Crowell model	This model is applied to those pharmaceutical dosage forms in which the dissolution of drugs takes place in planes parallel to drug surface, when the dimension of tablet diminishes proportional to the initial geometrical form with respect to time	It is complicated to use and understand in some cases

11.4 Factors Influencing the Rate of Kinetics in Kinetic Models' Selection

11.4.1 Mechanical Factors

The rate of transformation from one type of compound to similar or different type of compound can be significantly increased due to trituration and compression [24].

11.4.2 Physical Factors

The rate of kinetics can be affected by solubility and racemization of chemical compounds.

11.4.3 Relative Humidity

In the presence of moisture, transformation of both polymorphs to monohydrate form could be occurred.

11.4.4 Effect of Solvent

The presence of solvent vapors like dichloromethane can have significant effect on the rate of transformation.

11.4.5 pH

Acidic and alkaline pH affect the rate of kinetic reaction. The effect of pH either causes an increase or a decrease in the rate [25].

11.5 Conclusion

Kinetic models assist in describing the stability behavior with the help of stability studies as well as release patterns of drugs from different pharmaceutical dosage forms. The most common and simple models used are first-order and zero-order kinetic model. Higuchi model, Korsmeyer-Peppas model, and Hixson-Crowell model have a large implementation in the study of drug polymeric matrix system.

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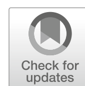

Stability Studies of Vaccines

12

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Abstract

Edward Jenner laid the foundation of vaccination as he was the first to introduce and use the procedure as well as pursued his scientific investigation against the deadly smallpox. Due to his work and the recent advancements in science, now the world claims that smallpox has been eradicated from the world. For long-lasting effective immunization, both the humoral- and cell-mediated parts of the adaptive immunity need to be stimulated. There are almost seven types of vaccines that are currently in use or in the stage of development. The benefit-risk profile (efficacy) of each vaccine is constantly evaluated during the entire process right from the individual component pre-clinical evaluation (in vitro and in vivo) and clinical (human trial) and developmental phase till the end use. The Vaccine Adverse Event Reporting System (VAERS) used by the Centers for Disease Control and Prevention (CDC) and Food and Drug Administration

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_12

(FDA) monitors post-licensure vaccine efficacy and safety using various methods and techniques. Advanced kinetic models are used to calculate the degradation kinetics rate of biological products such as protein and virus-based vaccines and also an emulsion-based adjuvant vaccine. Statistical tools are used to select an optimal number based on the variable parameters used for fitting of experimental data obtained from different steps of kinetic models.

Keywords

Vaccination · Smallpox · Humoral · Cell mediated · Kinetic models · Centers for Disease Control and Prevention

12.1 Introduction

For decades, smallpox had threatened the globe by causing countless deaths, for which the world is thankful to Edward Jenner (Fig. 12.1), whose innovative and remarkable contribution to immunization ultimately eradicated smallpox and also laid a foundation for the vaccine's development. Although China and India have used the principles of vaccination since thousands of years by inoculating live and

Fig. 12.1 Photo of the same boy prior and post smallpox infection. This is the reason why this disease was called variola (means stain or mark on the skin), speckled monster in the eighteenth century, and small pockes in the fifteenth century

virulent smallpox virus for later protection. However, Edward Jenner is honored as a pioneer of vaccine development almost 200 years ago, who gave it the name of *vacca* in 1798, which means cow [1]. From this first-ever development till the advancement of novel vaccines, vaccination has overwhelmed the consequences of catastrophic infections. Briefly, in the eighteenth century, the world had the vaccinia virus vaccine, and Louis Pasteur and Emile Roux in the nineteenth century explained that attenuated or inactivated organisms provide prophylaxis, and finally an accelerated advancement was observed to produce immunization using advanced technologies [2]. Today's research is focused to develop novel vaccines to defeat and eradicate deadly infectious diseases by boosting and engineering the immunity [3].

Vaccines are actually biological accumulation of antigens which are designed to provoke adaptive immunity by mimicking an infection to produce antibody and memory cells without causing illness [4]. There are different stages to measure vaccine's efficacy which starts from pre-licensure stage. A randomized controlled trial is carried out in which the disease incidence rate between non-immunized and immunized volunteers is considered as a direct effect of the vaccine [5, 6]. Estimation of vaccine effectiveness is usually measured from studies carried out post-licensure, which showed the actual protection against disease under real-life condition [5]. Both efficacy and effectiveness of the vaccines are calculated using the following formula:

$$VE = R_{\text{unvaccinated}} - R_{\text{vaccinated}} \div R_{\text{vaccinated}} \quad (R = \text{Risk or Rate})$$

Keeping in view this background, we will first shortly highlight the immune system and its types, short history of vaccine development, and types of vaccines to build a baseline study and then will focus on the efficacy and stability of vaccines.

12.2 Immune System

We are living in the world surrounded by an ocean of microorganisms called pathogens, both harmful and harmless to our body. These microorganisms in one way or the other get access to the body, select a predilection site, start multiplication, and thus harmfully affect the body. Nature has provided a protective screening system to the body, the main function of which is to screen these organisms at various stages and not allow them to enter into the body. In case they enter into body, the body has a defensive mechanism which first labels them as a foreign enemy and alerts the body's defense system to start a combat against them.

12.2.1 Types of Immune Systems

This body's defense system, which is named as immune system because of the immune cells involved in this process, can be divided into two main types: innate/general and adaptive/specific immune systems. Both of these types communicate

with each other to provide effective immunity to the body against the pathogens which gain access to the body.

12.2.2 Innate or General Immune System

The innate immune system is the first line of defense which provides the first-ever barrier against the pathogens trying to invade the body. This system provides a variety of protective measures which are continuously in function and are not specific to a particular pathogen; that's why it is termed as general immune system. Due to specificity of innate immune cells toward conserved molecular patterns, which are found only on microorganisms and not on the host cells, which prevents host cells from attack. This system lacks memory cells, so repeated exposure of the same pathogen limits the improvement of this system. Anatomical barriers (intact skin), mucous membranes, and acidic environment (pH 3–5) of the skin prevent the entrance and growth of pathogens. Mucus and cilia of the mucous membranes trap the microorganisms and propel them out of the body [7, 8].

Furthermore, physiological barriers such as the normal temperature of the body, development of fever, gastric acidity, lysozyme (hydrolytic enzymes found in body fluids), interferon (produced by virally infected cells), and collectins (surfactant proteins present in serum, lung secretion, and mucus) are also parts of the innate immune system, which can inhibit, eliminate, and cleave directly (disrupting lipid membrane) or indirectly by clumping the pathogens to enhance their susceptibility to phagocytosis [7, 9].

The complement system is divided into three pathways based on the way they are activated. Classical, alternative or properdin, and lectin pathways are activated when IgM/IgG antibody binds to antigen, when C3b complement protein is deposited onto the microbial surfaces, and when plasma mannose-binding lectin (MBL) attaches to microbes, respectively. These pathways merge into a single pathway which leads to the formation of membrane attack complex that forms pores in the membrane of pathogens. The complement system is also involved in the opsonization of a particular pathogen to direct it into phagocytosis and trigger inflammation. The inflammatory response with five cardinal signs toward an injury or pathogen is also a part of the innate immune system [10, 11]. Pattern recognition receptors (PRRs) such as pulmonary surfactant protein, C-reactive protein, Toll-like receptors (TLRs), mannan-binding lectin (MBL), C-type lectin receptors (CLRs), MYC-associated factor X (Max), and neucleotide-binding oligomerization domain (NOD) are membrane proteins found on all innate immune system cell's membrane. The PRRs recognize pathogen-associated molecular patterns (PAMPs) including LPS, peptidoglycans, lipoproteins, and flagellin and trigger cytokine release, opsonization, complement activation, and phagocytosis. PAMPs are present only on microbial cells and not on human cells [10, 12, 13].

Phagocytic and granulocytic cells are the final soldiers of innate immune system which links it with the adaptive immune system. Monocytes circulating in the blood and macrophages present in the tissues are the main cells of this mononuclear phagocytic system whose main function is antigen presentation, cytokine production, and phagocytosis [14].

12.2.3 Adaptive/Specific Immune System

The adaptive immune system has the ability to adapt itself when exposed and is also specific against a particular pathogen with memory for repeated exposure [10]. Humoral immunity composed of B cells and cell-mediated immunity composed of T cells are two parts of adaptive immune system [15]. B cells are produced in the bone marrow, processed, matured, and also exposed to the extracellular pathogen/toxins in the lymph nodes. B cells recognize pathogens with lipopolysaccharide, dextran, and bacterial polymeric flagellin (without being processed by an antigen-presenting cells) and become activated which is a weaker response. The response of B cells which are activated by T helper cells is much better with effective memory and is used for long-term immunization. The activated B cells are converted into plasma cells which then begin to produce specific antibodies. IgM is the first antibody produced followed by the antigen-specific IgG antibody. IgD, IgA, and IgE are also produced, but IgG is the most important antibody related to vaccines [1, 16].

Cell-mediated immunity comprises of T cells, which are mature in the thymus and are activated against intracellular pathogens. CD4 or T helper cells and CD8 or T cytotoxic cells are the two main types of T cells. T helper cells recognize MHC II (major histocompatibility complex), which is present in all immune cells, and therefore are called the markers of the immune cells. T helper cells are further divided into Th1 responsible for cell-mediated immunity and Th2 responsible for antibody-mediated immunity. T cytotoxic cells, which are responsible for cell-mediated immunity, can recognize MHC I protein, which is present on the body's nucleated cells without RBCs and thus are termed as markers for the body cells. Compared with B cells, T cells can only recognize the antigens which are being processed by antigen-presenting cells [1, 16].

12.2.4 Immunization and Its Types

It is a process by which the immune system of an individual is armed against the harmful enemy called immunogen. Broadly it can be divided into passive immunization by transfer of preformed antibody and active immunization by exposure of the body to pathogenic agents. Passive immunization is for a time being and is finished when the inducted antibodies are destroyed. In neonatal life, maternal antibodies that are transferred through placenta and to the newborn through colostrum and milk are natural ways of passive immunity. Gamma globulins and antivenin are examples of artificial way of passive immunization. Active immunization is when an individual is directly exposed to pathogen and the body starts developing a long-term immunity compared with a passive immunity. Active immunization can also be developed naturally (exposure to influenza) and artificially (different types of vaccines used). A viral antigen that bounds with MHC I protein and presented at CD8 will initiate cell-mediated immunity, while a bacterial or parasitic antigen will be bound on MHC II molecules and presented on CD4, which will trigger antibody-mediated immunity

[17, 18]. A list of the different kinds of vaccines which are currently in use or in the stage of development is mentioned in Table 12.1.

12.2.5 History of Smallpox and Vaccine Development

Smallpox is a viral disease believed to have appeared and destroyed millions of human populations in the early eighteenth century throughout the world. It was named as speckled monster in eighteenth century in England, variola (means stain or mark on the skin) in Switzerland in 570 AD, and small pockes in the fifteenth century. It has a unique history as it was the first disease for which vaccine was developed and which was also eradicated from the world [1, 19]. The survivors of smallpox were immune to the diseases, and many efforts were performed to cure the diseases and decrease the devastating effects as much as possible. Earlier, inoculation, derived from a Latin word *inoculare*, which means "to graft," was considered an efficient way to treat smallpox. In this procedure, a smallpox virus (a wet lancet with fresh smallpox matter) was subcutaneously inoculated into a non-immunized individual. Later on, this procedure was named as variolation, which became very popular and decreased the fatality rate up to 10 times [8, 20]. In 1756, a boy named Edward Jenner was variolated against smallpox who developed mild case of smallpox with subsequent development of immunity and survival. Edward had a strong interest toward science and nature during his school life, and at the age of 13, he heard about a dairyman saying that "I shall never have smallpox for I have had cowpox, I shall never have an ugly pockmarked face." In that time, it was a very common belief that the dairymaids are somehow protected from smallpox. In 1796 Edward Jenner concluded that cowpox gives protection against smallpox and could be transmitted with unknown mechanism from one individual to another. From a cowpox-infected person's lesions, Jenner inoculated the boy, and after subsequent mild signs, the boy recovered on the tenth day after inoculation. The boy was inoculated again from a fresh cowpox vesicle, but this time the boy did not develop any signs of the disease. In 1798, he published a booklet which was based on his experiments on variolation. The conclusion of this publication was that the origin of cowpox is from horses, which is then transmitted to cows, and cowpox infection provides protection against smallpox. He called this procedure vaccination and the fresh cowpox material as vaccine, which was derived from Latin words *vacca* for cow and *vaccinia* for cowpox. The vaccination replaced variolation and became popular in the early eighteenth century throughout the world [1, 21].

12.3 Quality Control and Vaccine Development

The achievements and benefits of vaccines to the world can be measured from various factors, including eradication of smallpox in 1977; polio, which was eradicated very soon; and reduction of deadly outbreaks and consequent mortalities. Several vaccines approved for human use are listed in Table 12.2. For the

Table 12.1 List of vaccines currently in use or in the stage of development with advantages/disadvantages and examples

Name of vaccine	Procedure	Advantages	Disadvantages	Examples
Live attenuated	Laboratory weakened version of live pathogen	Produce both strong cellular and antibody response, produce long-term immunity, lifetime safeguard, single- or highly two-dose inoculation	Possibility of reversion to the virulent form, cannot be given to individuals with weak immunity (HIV or cancer patients)	Smallpox, type 2 poliovirus, bacillus Calmette-Guerin (BCG), measles-mumps-rubella (MMR) vaccine
Inactivated or killed whole-cell vaccines	Destruction of the pathogen by heat, chemicals (formalin), or radiation; therefore cannot replicate in the live organism	More stable and protective, do not require refrigeration and can be freeze-dried for transport, safer with fewer side effects	Produce weaker immune response due to lack of replication and fast clearance from the body, additional booster doses required to boost immune system for long-term immunity	Influenza, hepatitis A, Listeriosis
Subunit (protein-based subunit polysaccharide-based subunit conjugates)	Contains only the antigen or epitope (antigenic part) of the pathogen; 1–20 antigenic parts of microbes can be used instead of entire pathogen	Most readily stimulate the immune system, specific and late adverse reaction, can be used by every individual	Difficult procedure to know which antigen should be included, no confirmation regarding forming memory cells	Hepatitis
				Influenza
				Streptococcus pneumoniae, *Neisseria meningitidis*, *Haemophilus influenzae type b*
Toxoid (bacterial inactivated toxins)	Produced by inactivated (detoxified) bacterial toxins by formalin with ddH_2O or sometimes with heat or radiation	Produced immunity against bacterial toxins, cannot get back to its virulent form, cost-effective	Produced immunity against bacterial toxins	Diphtheria, tetanus, pertussis, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b (Hib)
Conjugate	Epitope linked to polysaccharides	To invoke an immune response against the weak antigens (specially bacteria)	Specially used in infants	*Haemophilus influenzae* type B (Hib), *Streptococcus pneumoniae*, *Neisseria meningitidis*

(continued)

Table 12.1 (continued)

Name of vaccine	Procedure	Advantages	Disadvantages	Examples
Naked DNA	Injected genetically engineered DNA in the form of plasmid DNA (pDNA) encoding antigen	Can be produced relatively easily at low costs and to produce both humoral and cellular immunities; pDNA is stable at room temperature which renders cold chain requirement	Has been approved for human use, and various vaccines are in clinical trials and some are approved for veterinary use	Cancer and HIV-AIDS (under trial)

development of a novel vaccine, sufficient and exact knowledge of its protective mechanism, i.e., to provoke the immune system without causing illness, is needed for its quality control during the process of development [22]. However, there are certain constraints which need to be monitored to check its efficacy which is a laborious and continuous job. The International Conference on Harmonization (ICH) has developed pre-set criteria (various assays) to regulate the quality of a biopharmaceutical product on current good manufacturing practices (cGMP). To overcome adverse events and to get an overall acceptable level following immunization, risk assessment must be performed during each and every step of vaccine development [23]. The following are the important overviews of the major quality control issues which must be addressed before commercialization of vaccines.

12.3.1 Monitoring Efficacy of Vaccines (Pre-clinical Stage)

Vaccine is a unique complex formulation which is composed of one or more types of immunogenic components including live attenuated or killed viral or bacterial part, proteins, polysaccharide, polynucleotide, and conjugates. Apart from these, there are also some agents added to modify and boost a long-lasting immune response to the body. Vaccine efficacy is defined as the percentage of reduction in the incidence rate of disease and has minimal or no adverse reactions post immunization. So there is always a need not only to monitor the chemical and structural integrity of each constituent but also to ensure that the vaccine is capable of immunogenicity [24]. Safety assessment of vaccine starts from the pre-clinical evaluation of vaccine constituents (for purity, sterility, and stability), followed by the clinical and development phase, and finally the distribution, transportation, and duration of use of vaccines [25]. One of the most important factors is to maintain the shelf life of vaccine during the distribution process. For this bulk, intermediate as well as final container product also needs to be monitored for either maintaining the required

Table 12.2 Licensed vaccines for human use

Disease target	Causative agent	Trade name	Vaccine type	Vaccine components	Adjuvant used
Influenza	Influenza virus	AGRIFLU	Inactivated	Trivalent whole virus based (for types A and B)	None
		FluMist Quadrivalent	Live inactivated	Quadrivalent vaccine (for intranasal spray) contains an A/H1N1 strain, an A/H3N2 strain, and two B strains	
		Influenza A (H1N1) 2009 Monovalent Vaccine	Inactivated against H1N1 pandemic 2009	Monovalent split vaccine whole cell approach	
Tuberculosis	*Mycobacterium tuberculosis*	BCG vaccine "SSI"	Live attenuated	Bacillus Calmette-Guerin (BCG), Danish strain 1331	None
Anthrax	*Bacillus anthracis*	BioThrax	Subunit	Cell-free filtrates of microaerophilic cultures of a virulent, non-encapsulated strain of *Bacillus anthracis* and proteins including protective antigen (PA)	Alum
Cervical cancer	Human papilloma virus (HPV)	Cervarix	Subunit virus-like particle	Recombinant L1 protein, the major antigenic protein of the capsid, of oncogenic HPV types 16 and 18	AS04
		Gardasil		Recombinant quadrivalent vaccine prepared from purified virus-like particles (VLPs) of L1 capsid of HPV type 6, 11, 16, and 18	Alum
Hib-induced disease (pneumonia meningitis)	*Haemophilus influenzae* type B	COMVAX	Conjugate	*Haemophilus influenzae type* b conjugate and hepatitis B vaccine	Alum
Hepatitis B	Hepatitis B virus				
Hepatitis A	Hepatitis A virus	Havix	Inactivated virus	The virus strain (HM175 strain) is propagated in MRC-5 human diploid cells, treated with formalin to inactivate	Alum

(continued)

Table 12.2 (continued)

Disease target	Causative agent	Trade name	Vaccine type	Vaccine components	Adjuvant used
Polio	Poliovirus (types 1, 2, and 3)	IPOL	Live inactivated	Inactivated at +37 °C for at least 12 days with 1:4000 formalin	None
Diphtheria	*Corynebacterium diphtheriae*	Infanrix	Subunit	Toxoid of diphtheria and tetanus and the acellular pertussis antigens (inactivated pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin)	Alum
Tetanus	*Clostridium tetani*				
Pertussis	*Bordetella pertussis*				
Diphtheria	*Corynebacterium diphtheriae*	Pediarix	Subunit	As Infanrix in combination with HBsAg and types 1, 2, and 3 polioviruses	Alum
Tetanus	*Clostridium tetani*				
Pertussis	*Bordetella pertussis*				
Polio	Poliovirus				
Hepatitis	Hepatitis B virus				
Hepatitis B	Hepatitis B virus	Recombivax HB	Subunit viral	Derived from hepatitis B surface antigen (HBsAg)	Alum
Herpes zoster	Herpes zoster virus	Zostavax	Live attenuated	Oka/Merck strain of VZV	

temperature or not. Recalculation of the expiry date is needed after an unintended temperature excursion due to cold storage unit failure or mishandling during transportation [26]. All vaccines are prone to be damaged due to temperature elevation or freezing during distribution, during storage, and even during the use of vaccine [27]. The therapeutic agents (medicines, drugs, vaccines, etc.) and the medical or immunization procedures cannot be declared as totally risk-free but are used to ensure that the benefits are far much more than any potential emerging risk.

The final vaccine product is an ideal defined product (both in composition and structure) produced as a result of high-profile assays performed to monitor the efficacy of vaccine in order to reduce all the issues that are discussed in the above paragraph. The number and type of assays that must be performed depend on the type of vaccine to be prepared. For example, the most crucial assay for live attenuated vaccine development is to calculate the number of pathogenic particles that must be included in a single dose and the screening of impurities in each ingredient. When the product is licensed, the post-licensure test is carried out in the form of survey to know the efficacy and safety level of the vaccines when applied on a community [28]. To monitor the efficacy of vaccines during pre-clinical studies, essays are broadly divided into the following two categories.

12.3.2 Assays for Measuring the Concentration and Integrity of Antigens

To quantify an intermediate and final product as well as impurities in the final product, various colorimetric assays and analytical separation tests are performed. Calorimetric assays are used to measure the concentration of proteins and polysaccharide, number of primary amine groups and host cell DNA, etc. The techniques used to separate the impurity and maintain the integrity and stability of vaccines (protein and polysaccharide in nature) are chromatography (liquid and gas) and gel electrophoresis. Antigen characterization, impurity identification, structural degradation such as oxidation, and fragmentation can be easily measured by mass spectrometry [28]. A list of various physicochemical and immunochemical techniques, along with their details, is given in Table 12.3.

12.3.3 Assays for Measuring Biological Activity, Active Concentration, and Confirmation of Antigen

This part comprises of various assays designed for in vitro and in vivo studies as well as for physicochemical and immunochemical analysis. For the quality assessment of vaccines, both physicochemical and immunochemical evaluations are very crucial to confirm antigenic structure and access immune-dominant epitopes, especially for monoclonal antibody. The integrity of antigenic structure has an important role in vaccine potency and efficacy [29]. In combination vaccines, the quality assessment of individual antigen is a hurdle which must be proven by a correlate protection and

Table 12.3 Physicochemical and immunochemical techniques for vaccine development

Technique	Information regarding	Identity	Purity	Stability
Chromatography				
Reversed phase	Purity, stability, degradation, and protein modification	+	+	+
Ion exchange	Protein modification, degradation, aggregation, and purity	0	+	0
Size exclusion	Hydrodynamic size, aggregation, purity, and oligomeric repartition	0	+	+
Immunochemical techniques				
Biosensor analysis	Antigen concentration, epitope integrity, and binding kinetics	+	0	+
ELISA	Antigen concentration	+	0	+
Immunoblotting	Size, protein modification, degradation, and aggregation	+	0	+
Spectroscopy				
Circular dichroism	Secondary and tertiary structure	–	–	0
Electron microscopy	Imaging of supramolecular structures and integrity	0	0	+
Fluorescence spectroscopy	Tertiary structure of proteins and protein unfolding and refolding	–	–	0
Infrared spectroscopy	Excipients and protein structure	–	0	–
Light scattering	Aggregation	–	–	+
Nuclear magnetic resonance	Excipients and polysaccharide structures	–	0	–
UV absorbance spectroscopy	Tertiary structure of proteins, protein unfolding and refolding, and aggregation	–	–	+
Electrophoresis				
Capillary electrophoresis	Primary structure of proteins and protein degradation	0	0	+
Isoelectric focusing (IEF)	Isoelectric point (pI), protein modifications, degradation, aggregation, and purity	0	+	+
PAGE (SDS or native)	Size of protein, protein modifications, degradation, aggregation, and purity	0	+	+
2D electrophoresis (IEF plus SDS-PAGE)	Size, pl, protein modification, degradation, aggregation, and profile of protein impurities	+	+	+
Mass spectrometry				
	Primary structure of proteins, protein modification, and degradation	+	0	+
Differential scanning calorimetry				
	Thermodynamics of protein unfolding	–	–	+

+, yes; 0, neutral/possibly; –: no/unfavorable

efficacy both in in vitro and in vivo studies as well as immune chemical and serological studies [30, 31]. In vivo studies are a platform which provides an ideal outcome from immune response parameters including innate, cellular, and humoral immunity with protection. There are some restrictions especially to develop animal models for those human diseases which have specific range of surface proteins that interact with specific host receptors [32]. Keeping in view, it is acknowledged that the ability and safety of pre-clinical studies for the product which is human specific will be limited in the clinical trials. For this, transgenic mice with specific human receptors such as CD46 and CD66 have been developed which have now been used to develop novel human vaccines [33]. Similarly, humanized immune-deficient mice are also developed in which human tissues with immunological functions are transplanted into mice and studied [34].

12.3.4 Monitoring Efficacy of Vaccines (Clinical Stage)

To move from a pre-clinical stage (in vitro and in vivo) into a clinical stage (first human test) in vaccine development is anyhow a difficult decision. For this, the Committee for Medicinal Products for Human Use (CHMP) has developed certain guidelines which should be adopted before moving from pre-clinical to clinical trials [35]. The most important of these guidelines are some of the serious adverse reactions (discussed below) and their remedies, which must be kept in mind before moving to a clinical trial. To evaluate the efficacy of vaccines, serum antibody titer is checked in the clinical trials and is acceptable if it is above a certain threshold level. Similarly, cellular immunity measurement is also recommended to evaluate both quantitative and qualitative analyses of T cells, such as Th1, Th2, T memory cells, and relevant cytokines.

12.3.5 Adverse Events Following Immunization (AEFI)

Unlike medicines, vaccines are administered in bulk and mostly to the healthy population, such as children and infants, which possess low tolerance against potential risk or side effects of vaccines. As we have discussed, vaccines are composed of somehow a pathogenic agent which may evert and, apart from giving long-term immunization, may cause some discomfort and even death. As the name indicates, AEFI are the unwanted adverse events which occur after immediate or weeks after immunization irrespective of whether it is due to vaccines or not. Some of the AEFI are summarized in Table 12.4.

The Centers for Disease Control and Prevention (CDC) and the US Food and Drug Administration (FDA) are assigned to conduct and evaluate the safety as well as the efficacy of each vaccine post licensed, during various steps of distribution and after immunization [36, 37]. The CDC and FDA after evaluation submit the Vaccine Adverse Event Reporting System (VAERS) which is a passive (spontaneous) reporting system as shown in Fig. 12.2. The report is passively made based on the

Table 12.4 Various adverse events following immunization (AEFI)

AEFI	Details
Vaccines induced or vaccine reaction events	Some events caused post correct vaccination such as pain, swelling, redness, and even fever
	Inherent properties of vaccine such as presence of an adjuvant sometime causing local inflammatory response or eversion of live attenuated vaccines including mild fever and/or rash for about 10 days post immunization (MMR and poliovirus vaccines)
Immunization errors	Errors or mistakes made during preparation, processing, transport, handling, and administration of vaccines, e.g., injecting a fully liquid without reconstitution or administration of oral vaccine intramuscularly
Coincidental events	Immediate events followed by vaccination but not caused by vaccines such as flu-like symptoms, etc.
Immunization anxiety reaction	Anxiety reaction about or pain from the injection itself like syncope and panic attack after vaccination
Vaccine failure	Many reasons as mentioned right from the preparation till to the end users like failure in the cold chain requirements
Unknown	Difficulty to know about the cause

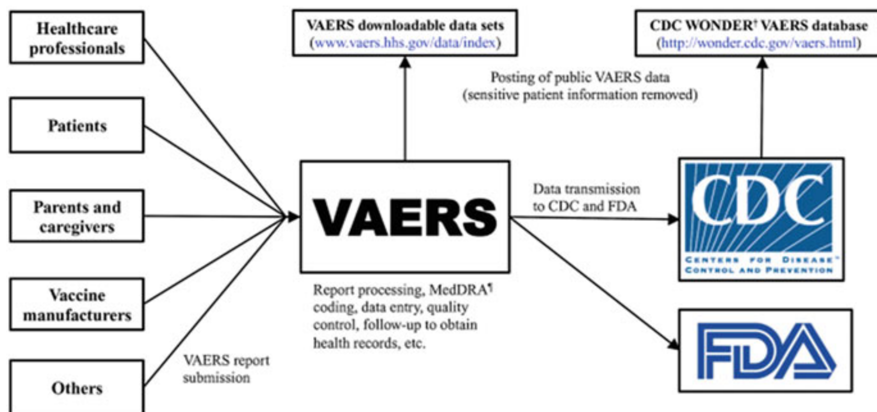

Fig. 12.2 Data flow diagram of Vaccine Adverse Event and Reporting System (VAERS) submission. During 2011–2014, healthcare professionals, patients and parents, vaccine manufacturers, and others (friends of parents, third-party reporter, etc.) submitted 38%, 14%, 30%, and 12% of reports (Adopted from reference [36])

volunteers who voluntarily share their experience, not based to identify and collect information. They also rely on the healthcare professionals, patients, parents, and caregivers and also on the health record surveillance registered system [38, 39].

12.3.6 Risk Factors Related with Vaccination

As highlighted in the above discussion, importance and benefits of vaccines could not be ignored, but there are various risk factors which need to be considered before immunization.

- Febrile convulsion, sometimes when a new vaccine is going to be introduced due to an identified risk for vaccines such as quadrivalent vaccines (e.g., measles-mumps-rubella and varicella vaccines).
- Spontaneous abortion risk, without identifying and investigating a potential risk prior to registration, such as when adjuvanted papilloma virus vaccines are administered without the knowledge of pregnancy.
- Immune-mediated disease risk, e.g., adjuvanted vaccination, such as adjuvant human papillomavirus vaccine and adjuvanted influenza vaccines.
- Immune-compromised pregnant individual, e.g., administration of adjuvanted vaccines to new target population where safety data is scarce.
- There is always a risk when introducing new vaccines, and the available infrastructure for pharmacovigilance is limited, e.g., for malaria vaccines and dengue vaccines in Africa and developing countries, respectively.

12.4 Vaccine Kinetics and Stability

Vaccine kinetics are typically used to determine the estimated degradation rates of vaccines using accelerated stability programs exposing the product to grater temperature than those recommended for storage of vaccines [40]. Immune system works against the pathogen and recognize pathogen characteristics. Therefore, characteristics of the pathogens are studied to increase the efficacy of vaccines, such as dimension of pathogens and specific PAMPs used as adjuvants for immune stimulation [41]. Vaccine stability is the most important step in vaccine efficacy and is a major concern associated with vaccine distribution. To study the stability of vaccine, the following two essential approaches are used.

12.4.1 Temperature Ramping Experiment

It involves the monitoring of changes that occur in biophysical properties of vaccine when the temperature is increased at a specific rate. These include monitoring the decomposition extent and/or some parameters related with thermodynamics including enthalpy and free energy. These experiments are performed very quickly (few hours) and are therefore used in vaccine formulation, development, and screening of stabilizing conditions [42, 43]. Besides thermodynamics, kinetics is also necessary to evaluate the stability of the vaccines. Kinetic stability is inversely proportional to the degradation rate, i.e., when the kinetic stability is high, the degradation rate is low and vice versa.

12.4.2 Accelerated Stability Studies

This study is performed to find out the degradation rate of vaccines over time because of exposure to high environmental temperature than the required recommended temperature for vaccine processing and storage. Liquid chromatography, gel electrophoresis, various immunochemical assays, and, to find out the antigenic titer, various methods are used to investigate the vaccine degradation rate at different time intervals [26, 44]. These studies are also used as precautionary measures to determine stabilized conditions, to enhance the potency of manufacturing process, and to estimate the shelf life of vaccines.

From the last couple of decades, medical field has made significant advances especially in the drug delivery system. The development of controlled release system is one of its great achievements. The main objectives of this system are to check the stability and provide the concentration of the vaccine in circulation or to the specific target site at a desired amount as far as possible [45], in a control release rate and time [46]. For vaccine stability, the controlled system initially releases the vaccine contents to attain the effective therapeutic concentration quickly followed by the release kinetics to provide the maintenance concentration. The main objective behind this is to increase the efficient availability, effectiveness, and stability and to release more effective product. So to find out and predict the kinetics of a vaccine, various mathematical models are used to compare all the phenomena that affect vaccine release kinetics [47].

12.4.3 Kinetic Model (Zero or First Order)

Kinetic model is the common accelerated stability model method used to analyze the data obtained and estimate long-term stability of a vaccine. To determine the constant rate for two or more temperatures, the data obtained from the accelerated stability studies are fit with a simple kinetic model, which is typically zero- or first-order kinetic models. Following experiment, an unknown constant rate is calculated from the data at a specific temperature using Arrhenius dependence of the reaction rate [44, 48]. Based on applications of zero- or first-order kinetics, stability estimation seems too simple for description of biological products, which commonly have complex and multistep degradation reactions [49].

12.4.4 Sophisticated Degradation Kinetic Models (Two-Step Model)

In this type of kinetic models, a two-step kinetic model is used such as an *n*th order and an autocatalytic component to investigate the degradation rate of biological products. Examples of this complicated process include Fulcher-Tammann equation or Prout-Tompkins nucleation models [50, 51]. This is a superior kinetic model which suitably mimics and investigates the complicated process of biological product decomposition [52].

Relatively the *n*th-order kinetic model is best fitted to calculate the degradation rate for an adjuvanted protein vaccine, two-step model for live virus vaccine, and an autocatalytic-type kinetic model for an oil-in-water adjuvant formulation [48]. Based on the experimental data and kinetic analysis, it has been estimated that there is approximately five percentage point difference from that of actual value for long-term storage conditions, post excursions of temperature, and during shipments of freeze-dried products [40].

12.5 Conclusion

So far prophylactic vaccination has shown a profound impact on public health sector by decreasing disease incidence rate; still there is a need of novel vaccine development to combat and defeat the novel infections. Safety and efficacy of vaccines are the major concerns which must be evaluated during pre-clinical (in vitro and in vivo), clinical (first human trial), and developmental trial phases to avoid adverse reactions following vaccination. This is quite a lengthy exercise which needs contribution from various research and developmental expertise, quality control and assurance, safe production, marketing, and sale. This chapter highlights various aspects and types of immunity and vaccines, different assays used to evaluate the safety and efficacy concerns, and various kinetic models to know the degradation rates of vaccines at different time points.

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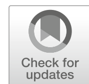

Stability Studies of Proteinous Compounds 13

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Abstract

Protein stability is a subject of great interest to the pharmaceutical, biotechnological, and food industry, as well as, for academic researchers who are studying proteins on regular basis. The stability of proteins is important to understand the optimization of protein purification, composition, expression, storage, and conformational studies. This study concentrates on the factors affecting the protein stability and type of stability undergone by proteins and chemical kinetics, which provide the designs and modifications of chemical reactors to maximize the product yield of pharmaceuticals and to eliminate hazardous by-products. A brief introduction of conformational and compositional protein stability will be discussed for analyzing protein stability by key methods. The importance of the different methods followed for pharmaceutical product stability testing, guidelines given by the ICH, FDA, WHO, and other agencies for stability testing, and other aspects related to pharmaceutical product stability will be discussed in a concise manner in the present study.

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_13

Keywords

Protein stability · chemical kinetics · ICH · WHO

13.1 Introduction

Proteins are huge, complicated molecules which have central importance in the body. They do much of the work and are necessary to build, operate, and control the tissues and organs of the body. In 1938, the Swedish chemists Berzelius coined the Greek term proteins for "protein," meaning "the first class."

Proteins are amino acid polymers. All proteins, either from the most primitive lines of bacteria or from the most diverse life types, are covalently bound to form linear arrangements of 20 amino acids in the same basic range. Each of these amino acids has a side chain with different chemical characteristics. Proteins vary according to amino acids that constitute the backbone of polypeptides differ in the shape, number, and sequence. Their molecular structures, biochemical features, and physiochemical properties are therefore differed. In many combinations and sequences, cells can generate proteins with distinctly different properties. The amino acid sequence defines the specific three-dimensional structure and function of the protein. They act as catalysts, hold and store oxygen and other molecules, offer assistance in defense system, nerve impulse, and transmission, stimulate movement, and regulate differentiation and growth.

A protein's amino acids have been categorized as aliphatic, aromatic, polar, nonpolar, hydrophobic, hydrophilic, oxidative, and acidic. For example, a protein may attain a regular secondary arrangement such as coils, α-helix, β-turn, and β-pleated board. The secondary structures are folded further leading to the construction of a higher cognitive structure called tertiary structure which is stabilized primarily by hydrophobic, van der Waals and electrostatic interactions, and hydrogen bonding. Proteins aren't rigid molecules entirely. They endure conformational variations after binding of ligands. When binding to oxygen, myoglobin and hemoglobin undergo structural modifications, and they can carry oxygen to the lungs and various tissues. Thus these proteins represent a relationship between structure and function.

In certain circumstances, exposure to denaturing conditions makes it possible to duplicate the structural transformations of normal functional proteins into misfolded, aggregated, or denatured proteins. The in vivo pathways of normal functioning proteins misfold into aggregates are still not well-known. How distinct proteins may structurally transform into a highly ordered aggregate structure such as amyloid fibrils which is extremely thermodynamically stable is unclear [49]. Well-known and etiological diseases were linked with the misfolding of protein in aggregates during the last decade. Alzheimer's disease and diabetes are among the most crippling, economically destructive, and expensive disorders in the developed world. As civilization ages, they become largely reliant on new agricultural and nutritional techniques [17]. It is important to consider the processes and concepts underlying the

folding of proteins, denaturation, and malformation in order to cure or potentially avoid these human diseases. This chapter will primarily concentrate on the functional modifications in proteins and their importance to product production stability. Further guidelines of proteinous compounds for drug stability will also be discussed.

13.2 Essential and Nonessential Proteins

Proteins are macronutrients supportive of body tissue development and maintenance. Amino acids are the rudimentary protein elements. Amino acids derived from poultry, beef, and legumes are essential, while amino acids synthesized naturally in your body are nonessential.

13.3 Protein Structures

The four types of protein structure (Fig. 13.1) are characterized by the level of complexity within polypeptides (Table 13.1).

13.3.1 Primary Structure

The basic order in which amino acids are combined together forms a protein primary structure (Figure 13.1a). Proteins are consisting of collection of 20 amino acids. In

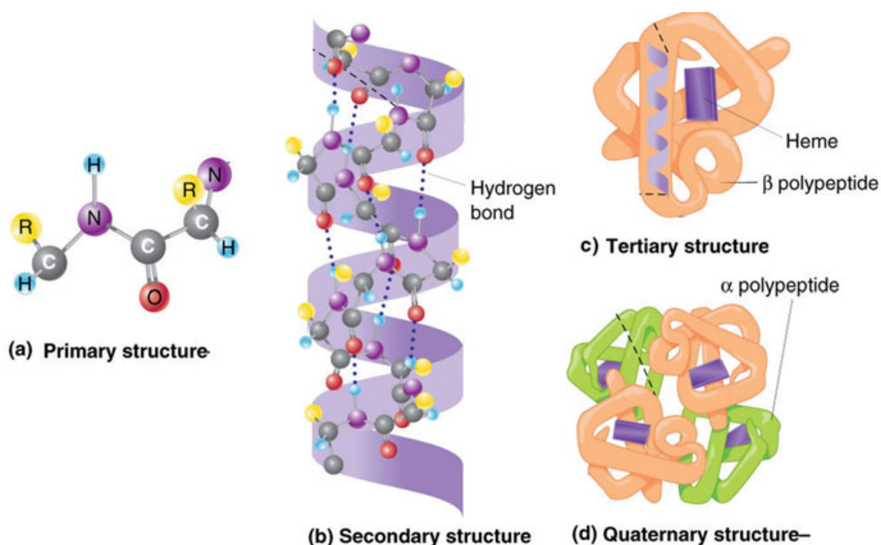


Fig. 13.1 Structures of protein

Table 13.1 *Common measures of protein stability*: Definitions of protein stability at each structural level are shown along with common methods used to analyze the degree of stability. Asterisks denote the relative merits of the three main structure determination techniques, with five asterisks denoting the optimal method. For example, NMR solution methods are often more favorable for studying dynamic processes and quaternary states as they are not influenced by crystal packing

Structural level	Definition of stability	Example biochemical processes or features	Common methods	Crystallography	NMR	EM
Primary	Primary change of amino acid sequence or modification of amino acids	PTM Proteolysis Protein splicing	Half-life analysis SDS-PAGE Mass spectrometry Eastern and Western blotting	*****	****	*
Secondary	Change of helix, sheet, and loop content	Secondary structure formation Racemization Aromatic side chain interactions Ligands	Circular dichroism (CD) Synchrotron radiation CD UV-CD FT-IR 2D-IR Deuterium exchange Mass spectrometry (DXMS)	*****	*****	*
Tertiary	Change of overall fold or protein Conformation	Hydrogen bonding Hydrophobic interactions Conformational change Disulfide bonding Topology	ITC DSC Thermofluor	****	****	*
Quaternary	Change in oligomeric state	Protein-protein interactions Oligomerization	Size exclusion chromatography Native gel electrophoresis	*	*****	*****

general, the following structural properties of amino acids have an amino group (-NH2), α carbon, an atom of hydrogen (H), a group of “unit” or “R,” and a group of carboxyl (-COOH).

13.3.2 Secondary Structure

Two kinds of secondary structures are present in proteins (Figure 13.1b). One is an alpha (α) helix structure, is identical to a helical cord, and is protected by hydrogen bonding in the polypeptide chain. The pledged layer beta (β) is the second form of secondary protein structure.

13.3.3 Tertiary Structure

Tertiary structure leads to the 3-D intrinsic polypeptide chain structure of a protein. Its tertiary structure contains several types of bonds and forces which hold a protein (Figure 13.1c). Hydrophobic interactions make a major contribution to a protein’s folding and formation. The “R” unit is hydrophobic or hydrophilic. Hydrophilic “R”-linked amino acids seek interactions toward aqueous environments. On other hand, the hydrophobic “R”-associated amino acids try to move toward protein center and away from the water.

13.3.4 Quaternary Structure

Quaternary structure means a macromolecule of protein containing several interactions between polypeptide chains. Polypeptide chains act as a subunit. Quaternary proteins may consist of more than one subunit of the same type of protein (Figure 13.1d). Example of a protein with a quaternary structure is hemoglobin. It comprises two alpha subunits and two beta subunits [53].

13.4 Domains of Protein

Most proteins are made up of some protein domains, i.e., protein sections which fold into distinct structural units. Usually, domains also have different functions. Binding modules, SH3 domain binds to proline-rich sequences in other proteins; kinase helps in enzymatic activities.

13.5 Classification of Proteins Based on Structure (SCOP)

The database Structural Classification of Proteins (SCOP) is a mostly manual classification of protein structural domains based on the similarity of their structures and sequences of amino acids. Setting the evolutionary relationship between proteins is a justification for this classification. Same shape proteins with no functional or sequence resemblance are put in separate superfamilies and are thought to have a shared very distant ancestor. Proteins with the same form and any sequence and/or function similarities are grouped in "families" and are believed to have a closer shared ancestor [59].

13.5.1 Chemical Composition-Based Classification of Protein

Based on their chemical structure, proteins can be divided into two groups (Fig. 13.2)

13.5.2 Simple Proteins

They are also known as homoproteins and consist only of amino acids. Examples include albumin, keratin, collagen, and plasma.

13.5.3 Conjugated Proteins

These are sometimes called homeoproteins; they contain a nonprotein component in their structure. Phosphoproteins, glycoproteins, and chromoprotein are examples of conjugated proteins.

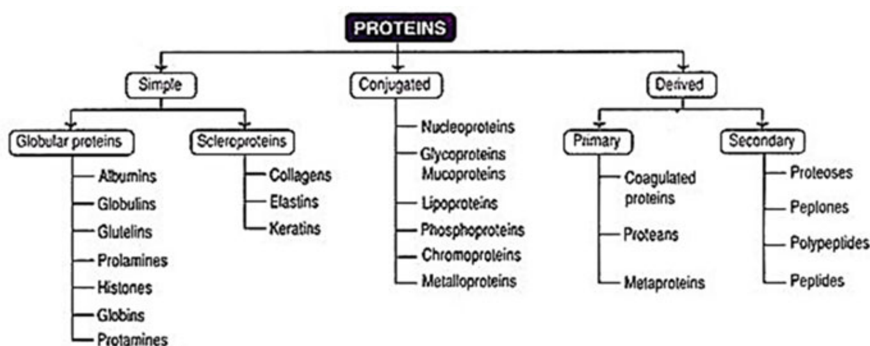


Fig. 13.2 Classification of proteins based on chemical composition

13.5.4 Phosphoproteins

These proteins bind the threonine and serine residues to phosphoric acid. They usually have a structural function, e.g., phosvitin of egg yolk, tooth dentin, and caseins of milk or reserve function.

13.5.5 Glycoproteins

These proteins are bind to polypeptide backbone with one or more units of carbohydrates covalently. The divisions typically comprise of more than 15–20 carbohydrate units comprising arabinose, galactose, 6-deoxygalactose (fucose), glucose, mannose, and N-acetylneuraminic acid (Neu5Ac or NANA). Examples are:

(1) Fibronectin binds the extracellular matrix to cells by interactions with fibrous or collagen proteins on one side while cell membrane on the other side.
(2) The best-known example of erythrocyte membrane glycoproteins is glycophorin.
(3) Immunoglobulins or antibodies and all plasma proteins in the blood, except albumin are the best examples of glycoproteins.

13.5.6 Chromoproteins

These proteins are containing colored prosthetic group. Myoglobin and hemoglobin binding with four and one class of hemes, respectively, rhodopsins binding retinal, and chlorophylls binding a porphyrin ring with its central magnesium atom.

13.5.7 Classification of Proteins According to Shape

13.5.7.1 Fibrous Proteins

These proteins have primarily structural and mechanical functions, serving cells and the entire organism. The hydrophobic amino acids, both internally and on their surface, make these proteins soluble in water. In supramolecular structures, hydrophobic amino acids are present on their surface and make their packing simpler. Here, the long filaments or sheets of polypeptide chains in most instances can only be found and repeated in one type of secondary structure. The structural properties of these proteins actually preserve their stability, and they provide shape, support, and protect vertebrates. These proteins are partially hydrolyzed in gut-like, alpha-keratine. Some examples are given here.

13.5.7.2 Fibroin

It is made of insects and spiders. An example of this is the silkworm developed *Bombyx mori*.

13.5.7.3 Collagen

"Collagen" does not mean a single protein but a group of structurally linked protein (at least 29 different types), which is the common extracellular scaffolding of multicellular organisms and dominant protein in the connective tissue. It comprises around 25–30 percent of all proteins in vertebrates. They are found in high concentration in eye cornea, cartilage, organic bone matrix, and tendons and other various tissues and organs. They shape various structures of the tissue, each able to fulfill a specific need. For example, these are enticed and guided fibers in the skin, which maintain the strength of skin tensile. The molecules are in crystalline position in the cornea, so that they are almost transparent.

13.5.7.4 α-Keratins

Almost all dry weight of skin's outer layer, claws, beaks, nail, wool, fur, and hooves is made up of α-keratins. The specific rigidity and flexibility of these structures result from the number of disulfide bonds which contribute to the stabilization of the protein structure along with other binding forces. And this is why, in beak and claws, keratins are rich in disulfide bonds, while wool keratins are smooth, extensible, and flexible because of less disulfide bonds.

13.5.7.5 Elastin

The skin and blood vessels possess elasticity due to random coiled structure of elastin; it varies from α-keratin and collagen structures. .

13.5.7.6 Globular Proteins

Most of the proteins belong to globular family. These are more complex and spherical than fibrous proteins and have a compact construction. These are normally water soluble but can also be present in biological membranes that result in a hydrophobic environment (transmembrane protein). Globular proteins act like, hormones, enzymes, membrane conveyors and triglyceride conveyors, receptors, blood oxygen and fatty acids, antibodies or immunoglobulins, and legume protein and grain. Most animal-associated globular proteins are solubilized to amino acids almost entirely at the intestinal level.

Cytochrome c, myoglobin, and hemoglobin are examples of globular proteins.

13.5.8 Classification of Proteins According to Solubility

Proteins are categorized into the following categories based on their solubility in water

13.5.8.1 Globular Proteins

They're soluble in water. They include the proteins that are functional, such as hemoglobin, enzymes, etc.

13.5.8.2 Fibrous Proteins

These are insoluble liquids and comprise the structures of proteins. They are protective (e.g., fibrin and hair keratin) and/or supportive (e.g., collagen).

13.5.9 Protein Classification Based on Functions

Schematic representation of classification of proteins on the basis of their function has been described in Fig. 13.3.

13.5.9.1 Hormonal Protein

Hormones are protein-dependent chemical compounds that are secreted by cells in the endocrine gland. Usually blood-borne hormones act as chemical messengers to relay signal from cells to cells. They affect numerous target cells in your body. Such cells have special receptors linked to signals by the hormone. Insulin, which secreted by the pancreas to regulate the blood sugar levels, is an example of a hormone protein.

13.5.9.2 Enzymatic Protein

Enzyme proteins facilitate metabolic processes in your cells, including stomach absorption, liver functions, glucose conversion, and blood clotting. One example is digestive enzyme which breaks the food down into simple forms which your body can easily absorb.

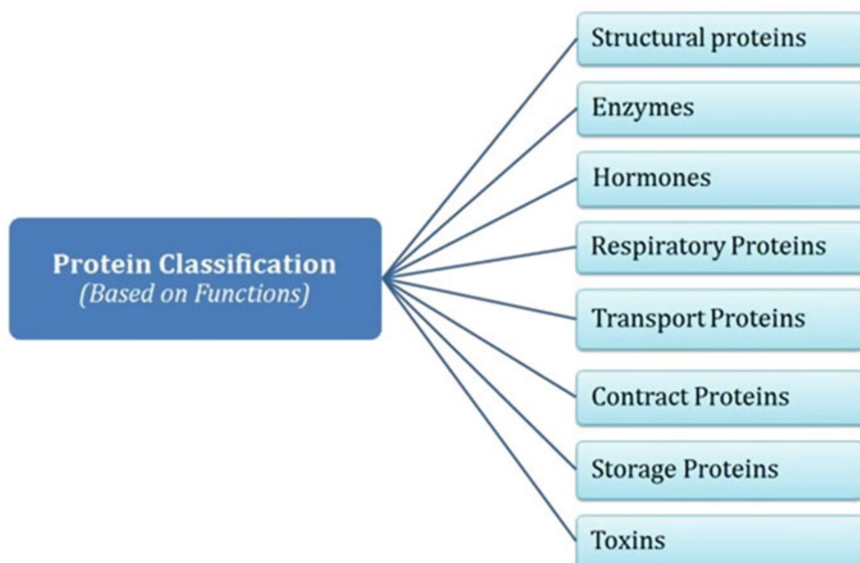


Fig. 13.3 Classification of protein on the bases of function

13.5.9.3 Structural Protein

The structural proteins of the body are also known as the fibrous proteins, which include elastin, collagen, and keratin. Collagen as a connective structure shapes the cartilage, tendons, bones, muscles, and skin. The nails, teeth, hair, and skin are mainly components of keratin.

13.5.9.4 Defensive Protein

Central parts of the immune system are immunoglobulins or antibodies, which carry a loop for diseases. In WBCs, antibodies are produced which targeted viruses, bacteria, and various other microorganisms and make them inactive.

13.5.9.5 Storage Protein

Proteins in the body mainly store ions including potassium in the mineral. Casein and ovalbumin, for example, are storage proteins found in breast milk and egg whites that play an important role in embryonic development, respectively. Iron is an ion needed to produce the main structural component of red blood cell, hemoglobin. Ferritin-protein storage controls and protects the body from adverse effects of excess iron.

13.5.9.6 Transport Protein

Transportation proteins provide the cells with important materials. For example, a transport protein, calbindin, facilitates the calcium absorption from intestinal walls. Serum albumin contains blood fats. Oxygen from the lungs into the tissues is transported by hemoglobin, while myoglobin absorbs oxygen from hemoglobin and transport that oxygen the muscles.

13.5.9.7 Respiratory Protein

These are colored proteins. All of them are conjugated proteins, and they contain pigments (chrome) as their prosthetic group, for example, hemoglobin and myoglobin.

13.5.9.8 Receptor Protein

Substance like nutrients and water that can enter and leave the cells are regulated by receptor proteins on external cells. Some receptors activate enzymes and glands. Endocrine gland stimulates epinephrine and insulin to regulate blood sugar level.

13.5.9.9 Contractile Protein

These are the motor proteins that control the speed and frequency of contractions in the muscles and heart. These contractile proteins are myosin and actin. Extreme contractions may cause heart problems (https://www.tuscany-diet.net/proteins/classification).

13.6 Stability of Proteins

The net energy balance which determines whether a protein is its original, folded or denatured (unfolded or prolonged) condition is protein stability. The net protein stability is extremely limited and is a contrast between two major contrasting forces. Various interactions (atomic / group), such as electrostatic, Van der Waals, disulfide, hydrophobic, and hydrogen bonding, stabilize the folded natural form of protein structures and regulate free entropic and non-entropically energies. In view of the relatively high complicated three-dimensional structure and rapid spontaneous folding of proteins, net stability is surprisingly low, usually between 5 and 10 kcal / mol. [72]. The word stability can be defined as the tendency of the protein structure to maintain the native (biologically active) conformation. Local proteins stabilize only slightly, shift to free energy (Δ G) in the 20–65 KJ/ mol range, separating plied and unfolded states under physiological conditions in typical protein.

Theoretically, a specific polypeptide chain may be able to take various configurations and thus describe the deployed protein condition with a high degree of confirmatory entropy. This interaction between entropy and hydrogen connection maintains the unfolded state in multigroup polypeptide chain. These effects revert and stabilize the folded native conformation by means of chemical interactions involve strong bonds including (non-covalent) peptide and disulfide bonds, hydrogen bonds, hydrophobic, and ionic interactions. 200–400 KJ/mol are required to break a single covalent bond, whereas a mere 4- KJ / mol can disrupt weak forces. Each covalent bond that results in native protein conformation such as disulfide bonds linking different parts of a single polypeptide chain is apparently much stronger than specific weak interactions. The protein conformation is the one that typically has the lowest free energy and the highest interplay intensity. Not only are there several weak interactions among the various molecules (intermolecular interactions), but they are also performed in just one molecule. Overall, intermolecular interactions are much less active than intramolecular. The stability of proteins isn't just the amount of free energy that comes from its many weak interactions. Inside a folded polypeptide chain, a hydrogen bonding group is bound to the water before folding, and with each hydrogen bond formed into a protein, a hydrogen bond (with equal strength) is broken between the same body and water.

In determining the net thermodynamic contribution of hydrogen bonding to folding, energy required to split the water hydrogen bond is distinguished from that extracted energy in folded proteins between two atoms in the formation of hydrogen bonds. The net stability that leads in the folded and unfolded states to a certain weak interaction or difference in energy is almost zero. However, that the power of a hydrophobic linkage is not due to strong intrinsic appeal between nonpolar groups. It is also important to note that the water solvent properties in which nonpolar groups are dissolved are the key cause for this interaction. A nonpolar residue dissolved in water creates a solvation shell in water that strongly order water molecules. As the polypeptide chain is folded into two nonpolar groups, the exposed surface area to solvent is decreased, and a portion of water highest order is released into the bulk solvent in the solvent shell. Entropy of water is also on the

rise. Entropy increases the driving force behind nonpolar solvents and enhances thermodynamically favorable process. In a protein, tightly packed core side chains of hydrophobic amino acid are usually inside. In aqueous solution, proteins enclose water and swell. As protein solution charged, they will colloidal emulsoids or micelles, and there is ring around each molecule. When a polypeptide loses its superior structure, denaturation of protein occurs which tends to minimize the biological activity.

13.7 Types of Stability

A structural biologist uses techniques such as cryo EM, crystallography, or NMR spectroscopy for evaluation of precise molecular structure of protein. A significant challenge to the crystallization of certain proteins is the reliance on crystallographic science, such as lack of or protein stability. Protein stability is a wide field addressing physical and chemical elements, entropy, thermodynamics, computational chemistry, dynamics, and protein folding [13]. Stability is essential to understanding the evolution of biological processes. It is the capacity of a sequence to persist over time [82]. Apparently, the particular nature of a protein molecule's "pattern" is rather unclear, but this "pattern" can turn by processes such as unfolding protein, degradation, denaturation, enzymatic alteration, conformation shift, and proteolytic cleavage. As regards the quality of the fully folded protein's primary and conformational structures, these transformations are usually considered or analyzed. Additionally, the stability of proteins means different things for different individuals. For example, the half-life activity of a protein may primarily be considered to be a test of its stability by a pharmacologist, biotechnologist, or food science. A protein chemist or a structure biologist, however, may use adjustments in all levels of protein structures as a test of its stability. Stability of proteins is a basic precondition for crystallization. At this stage, distinction between thermodynamic stability and conformal disorder of protein is important to consider, especially provided some specific parameters used for the characterization and analysis of structures by structural biologists.

For instance, NMR spectroscopy experts often note dramatically divergent values within their ensemble structures of a given protein crystal model, whereas crystallographers are estimating positional uncertainty by documenting factors B. Each of parameter defines displacements and disorder within a system which may show the degree of conformation stability. Systemic genomics projects have much alleviated the bottlenecks of out mode systemic, but even researchers are too mindful to deal with difficulties of encoding the puzzling protein target and purifying it. The primary structure, architecture configuration, and expression conditions of host cells should all be taken seriously to reduce much of the problems observed in stability of protein like purification and expression. Several specific approaches will address on protein stability tests, including the analysis of the protein melting temperature (Tm), cryo-EM, and NMR strategies that are widely used to maintain proteins' stability.

13.7.1 Stability: An Important Precondition for Crystallization

The self-organization of macromolecules into a long-range, translational periodical structure can be described as biomolecular crystallization. To accomplish this function effectively and efficiently, the moieties of a crystal's asymmetric unit should have the similar kind and shape. A central and prevalent criterion for crystallization of a protein has not been fulfilled if these stable objects can't form, and no attempt is made at discovering appropriate kinetic and thermodynamic circumstances leading to these protein composition crystals.

13.7.2 Compositional Stability

In the crystallization experiment, the same species must be maintained during the crystallization processes; some sort of compositional stability must exist. This ensures that on a basic level, the protein molecules have to have the same chemical composition. A sample's chemical uniformity can also be calculated with mass spectrometry or an SDS-PAGE gel. The primary structures of proteins are usually impairing due to post-transitional changes like proteolysis and glycosylation, which generate compositional heterogeneity. As it takes time to crystallize the protein, the primary requirements of compositional stability should be preserved by a given duration and an appropriate variety of environmental circumstances. It is worth noting that absolute stability is nothing like that. For example, Protein compositionally stable is enough to produce SDS-PAGE gel single band for crystallization experiment which can still not stable enough over a time.

13.7.3 Conformational Stability

If the protein sample is compositionally uniform, it therefore would also not crystallize until it is stable conformationally [80]. A large number of conformationally disordered proteins are considered to be exhibiting less or no organizational conformation. A protein with major abnormalities or regions exhibiting complex variability is less able to autonomously shape a crystal [60]. This could be true only though the composition of the sample is completely stable. The rigorous criterion for minimum conformational variation is a specific challenge while seeking a sample of protein to crystallize confronting a crystallographer. The puzzling problem is that a context-driven property is the conformation of flexible protein regions. Conformations can be somewhat specific in a macro-molecular crystal, for example, in an NMR. Although it is important to remember that the findings may only be applicable in relation to the nature and circumstances of such a specific methodology, structural approaches are used to evaluate conformational homogeneity. For example, the studies of conformational stability and dynamics, as crystal packaging can impede these motions, are sometimes limited using crystallographic methods. Complementary details can be given in such cases by NMR solution methods. By X-ray

crystallography, structures assessed provide scant evidence about the protein structure dynamics. Nevertheless, the atomic model contains some complex knowledge in the style of the factor B or atomic displacement parameter (ADP).

The factor B functions as statistically reflects atom's probability on the structure at mean specific location, and it is presented in A°2 [110]. When a given atom's B factor is high, then it means that the likelihood of having the atom in the structure at that location is small. In crystals, high-B-factor regions atoms may be moved because of complex disturbances in the polypeptide chain or a long or short-range disruption. Such dynamic or flexible regions can also be formed in a crystal and planned for better conformation stable protein samples during cloning. These improvements will not only help to improve the stability by purification and expression but also raise the likelihood of molecules being able to stack more organized in the crystal lattice. Consequently, these efforts also result in improved diffraction of crystals and X-ray data with higher resolution. In root-mean-square variance between the corresponding atoms of the members of the ensemble, it is possible for a comparison of structures produced by NMR spectroscopy to obtain a measure close to a crystallographic factor of B. This calculation can be used to determine the heterogeneity of stability, disorder dynamics, or stereochemistry through a range of conformational designs. The crystallographic factor B is equivalent to the meaning r.m.s.d. and is not disrupted or affected by crystal packing because the structure is in solution [85].

A broad class of proteins, called IDPs, includes large amounts of confirmatory disruption and, in some instances, has no apparent three-dimensional structure overall. 40% of the human protein is considered to have a minimum of one section, and 25% is disorganized fully [96]. Owing to its predicted problems in crystallization, the crystallographic group has fully prevented such proteins. However, NMR techniques were used to reveal the functions of these unstructured proteins [111]. Recent experiments introduced a profound shift of protein function and structure. Modern theory suggests that protein works in a lock and key manner which binds to ligand (or protein) by adopting a pre-shaped rigid structure. However, the NMR studies of Mujtaba et al. [68] and Sugase et al. [93] on p53, 14–3-3 and CREB (IDP proteins) indicated that these disordered territories provide for plasticity and versatility and often shape structure only when the partner protein is binding. These core proteins interact in sensitive context with several protein patterns, which is a feasible way due to initial loss of conformational stability and elasticity [102]. These large "central protein" complexes will have high-resolution crystal structures necessary to understand their role in human diseases such as Alzheimer's and Parkinson's diseases. While the low conformable stability of such protein poses problems, cellular IDPs provide several benefits relative to more conventional single structure folded proteins [56]. Besides IDPs, some proteins include APRs, which typically include 5 to 15 amino acids that appear to shape expanded board structures. Their usage is not restricted to IDPs. APR parts present in macroglobulin are responsible for amyloidosis disorders due to aggregated amyloid fibers [3]. Another protein named IIPs cannot be replenished with conventional buffer solution and is completely insoluble. Normal V22-SH3 and Mutant SH3 are resurrected and

solubilized in pure water but intractable in the presence of ions, allowing NMR spectroscopy to study further unstructured proteins in solution [36, 57].

13.7.4 Structure Level Stability of the Proteins

One clear approach to conceptualize protein stability from a theoretical point of view is to find stability at increasing protein structure level.

13.7.4.1 Primary Structure

Post-translational modifications (PTM) severally modified the sequence of primary protein or amino acid in peptide chain. PTMs are the central debate of protein stability which results in alteration of function and structure of protein. Conformational and compositional stabilities can be affected by instability and dynamic of protein and can be in complete or defective, respectively, by PTM. This freedom of conformation contributes to increased disorder, thus intermolecular crystal formation preserving the charged residues on the surface of protein. Although the heterogeneity of glycosylation appears to delay crystallization, its divergence may have significant functional consequences for a protein. Proteolysis, protein splicing, and inclusion of other function group of amino acid arise PTMs in the primary structure of protein. These types of PTMs modify the enzyme's activity and specificity and directed a wrong protein to particular area. For example, ethanolamine phosphoglycerol, hypusine, and carboxylate are the functional groups used to regulate protein operations [75, 100, 105]. Isoprenoid, palmitate, myristate, and glycosylphosphatidylinositol (GPI) are functional groups which often used for membrane targeting by attaching itself to proteins [10]. ISG15 [61], SUMO [39], ubiquitin, and PUP [92] are large peptides which can covalently bound to proteins.

Many PTMs play role particularly regard the protein's half-life and turnover within the cell, important for protein stability. PTMs include acylation, butyrylation, ADP-ribosylation, malonylation, iodination, succinylation, s nitrosylation, S-glutathionylation, glycosylation, and oxidation. All of these PTMs have vital role in the function and structure of target protein. Proteolytic and protein splicing like certain PTMs have a significant effect on the primary level of protein structures and lead to dramatic alterations in the compositional stability. A polypeptide precursor is processed to form a functional and mature protein. An auto-catalytically excised protein precursor is intein, which is of particular interest to protein stability, and they are also concern for a protein engineering point of view [2]. Sniping exteins are used to produce two new peptides ligated together [66].

13.7.4.2 Secondary Structure

Secondary structure of protein is a chain of tridimensional compact structure of polypeptide. In terms of stereochemistry pattern, polypeptide backbone of carbonyl O atom and amide H atom between hydrogen bonding describes the secondary structure [76]. The principal hydrogen bonding and hydrophobic behaviors are fairly

behind the formation of a secondary structure and in turn tertiary structure at simplified stage. -helix is the primary element of a secondary structure containing around 1/3 of all components. Residues including leucine, glutamate, and alanine are typically present in helices when the first crystal structure was examined [91]. By comparison, glycine, aspartic acid, and proline were detected less gradually. According to popular method of Chou and Fasman [12], many algorithms for protein secondary structure prediction were used to construct. The destabilizing impact of proline on helix (G 3.16 kcal/ mol vs. alanine 0.1 kcal/ mol) is a dominant consensus in the amino acid trends. This is a consequence of the absence of the amide H atom in the backbone that averts proline from contributing in the stability. Due to steric hindrance, proline thick cyclic side chain of 30 percent kink in backbone helix is obtained.

Glycine has the second lowest tendency to shape -helices due to greater strength of conformation when folding to shape α –helix. It's necessary to know all such propensities of are context based [40]. Proline, for example, is very popular in integral membrane protein transmembrane helixes and under these conditions is stable for helixes [55]. These findings clearly support the hypothesis that the folded protein stability is mainly determined by the composition of the amino acids, and the primary design results as a normal, realistic minimum of free energy. Easy algorithms for the construction of both stable helices and sheets have been applied to these simple principles [50]. Relative modeling can also be used to construct thermal stability proteins that are of a higher level, and associated models can be used to describe the tertiary structure.

13.7.4.3 Tertiary Structure

Overall folded form of polypeptide chain of protein is tertiary structure of protein. Primary and secondary structure, composition and conformation, cellular environment factors, pH, temperature, solvents, salt bridge, ligand binding, hydrogen bonding, van der Waals forces, hydrophobic forces, cofactors, ion bindings, PTMs, and chaperone are some factors influencing the protein folding process [16]. Many of these effects have recently been quantified by a series of Pace and colleagues experiments [74]. Hydrophobic reactions contribute 60% to protein stability and 40% to hydrogen bonding [73]. By conformational entropy, a single methyl group loss 2.4 kcal/mol to net instability and 1.1 kcal/mol to net stability of protein [73]. The net input of hydrogen binding to the total safety of proteins stability is 1.1 kcal/ mol 1. In addition, hydrophobic reactions normally do nothing for small protein stability [74]. For thermally protein stability for industrial usage, for example, proteases and biofuels for washing detergents of the shielding fold are particularly critical.

The deep hot vents of the sea Sargasso need protein, in those extreme conditions, in order to maintain the fold and structure of thermophilic species such as *Thermotoga maritima*. A thermophilic protein analysis indicates that protein compositions are essentially mesophilic, and small variations in amino acid composition imply thermal stability. There are some anomalies as applied to thermophilic proteins, including an upsurge in salt bridges, hydrophobicity, and aromatic deposits

[31, 113].Unlike the IDPs, the integrity of the tertiary protein structure is also treated as important to preserving protein function. However, as part of its mechanism of action, several proteins experience complete alteration in protein fold [104]. For example, serpins when interacting with the proteinase, endure a change after a longer, stable native stressed (S) to a more compact folded shape relaxed (R). These structural reorganizations include inserting a ring in core sheet or in the strand to generate a dimer that can cause polymerization [112]. Big conformational modifications like this are normal and are found in other proteins including lymphotactin, HA influenza, intracellular channel chloride LIC1, and protein at the Mad2 spindle checkpoint. It is therefore critical that every examination of stability takes careful account of the structure of the protein being studied, as certain protein folds are engineered to be unstable inherently [7].

13.7.4.4 Quaternary Structure

A quaternary arrangement is the organization into a multi-subunit matrix of the subunits of folded proteins. The stability of the complexes is crucial in regulating allostery and cooperation, often the product of the improvements in conformation in each polypeptide chains. The Monod–Wyman–Changeux (MWC) model is one of the standard models to explain protein's allosteric transformations [67]. In this model, there are two states in which protein exist: one is (R) relax, and other is (T) tense. Ligands can be bound to T or R any one state, but if bound with R, the affinity of protein will be improved. If ligands are attached to state T, affinity is decreased, and an allosteric modulator substance is established. Hemoglobin, deoxyhemoglobin in the state of R, and oxyhemoglobin in the state of T, are the possible candidates [6, 78].

As discussed above, recognition of protein stability in isolation from action is not appropriate for IDPs and metastable proteins [71]. Many proteins experience major conforming changes affecting both the secondary and the tertiary structures, and the compositional or conformational stability of each state is different [35, 103]. Abbott drug Navitoclax is an example of destabilizing, which destabilizes the relationship between the Bad / Bid / Bak and Bcl-2 an antiapoptotic protein. Roche drug Nutlin-3 is another destabilizer which inhibits interaction between MDM2 and p53, two tumor suppressors [42]. Cyclosporin A is a natural product, which stabilizes the bond between cyclophilin and calcineurin and increases protein-protein interaction. Tafamidis, a synthetic product, binds to transthyretin packet at dimer boundary are the antidote of destabilization [84]. Tafamidis stabilizes the transthyretin dimerized component and prevents the aggregation and misplacement seen with transthyretin amyloidosis disorders [8] as shown in Fig. 13.4.

13.8 Factors Affecting Protein Stability

The optimal requirements for keeping each human protein stable have to be empirically determined. Protein solutions are, however, usually kept cold (< 4oC) for experiments and other processes involving different temperatures. Several proteins

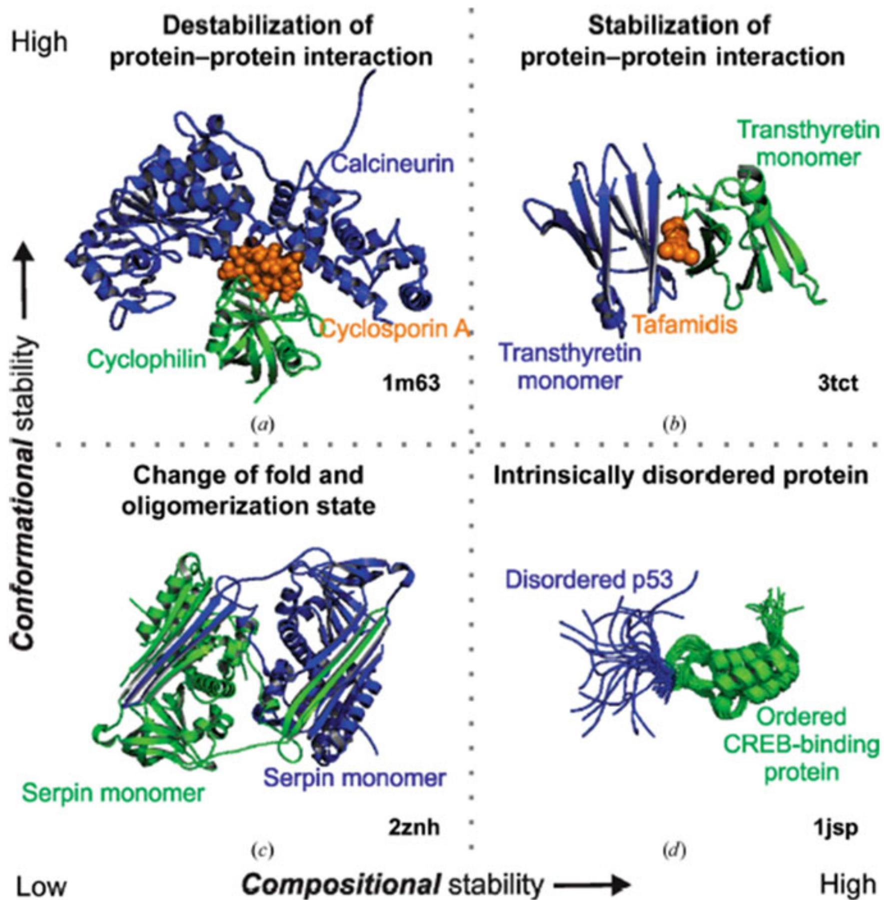


Fig. 13.4 *Matrix of examples of protein stability and disorder*. (**a**) Examples of proteins with high conformational stability include the protein-protein destabilizing compound cyclosporin in complex with calcineurin and cyclophilin, (**b**) the protein-protein stabilizing drug Tafamidis in combination with transthyretin, (**c**) examples of proteins with low conformational stability include the serpins, which undergo large changes in fold and oligomerization state, and (**d**) intrinsically disordered proteins (IDPs) such as the tumor suppressor protein p53

are highly suggestible which should be kept at −20 °C or − 80 °C. Moreover, continuous freezing and defrosting of protein solutions are also damaging. For the preservation of buffer, glycerol (50%) is added which lowers the freezing point and enables storage at −2 °C. Protein solutions can also comprise proteins such as heavy metal chelating agents and/or antioxidants. During cell destruction, proteases may be emitted, and thus protease inhibitors may need to be used.

13.8.1 Temperature

The unfolding free energy of a protein is defined as thermodynamic equilibrium, and it is calculated as differential equilibrium by its folded and unfolded positions. Therefore, knowledge about structural and functional dimensions that contribute to the stabilization of unfolded conditions is important in order to understand the diverse interconnectedness between enthalpic and entropic contributions to the stabilization of proteins. Latest experimental findings suggest that unfolded protein states are arrays of quickly interconverting, structurally, and dynamically distributed intermolecular interactions that can be relatively compact and exhibit significant nonrandom structure quantities. Leftover the secondary and tertiary structure are maintained in unfolded positions relating various long-term interactions, some are native, and many are found in the (folded) natural state. Temperature variations will break the hydrogen bonds and hydrophobic interactions. The explanation is that kinetic energy is increase by heating that the bonds are broken deeply by quickly wobble the molecules. This is one of the reasons in the use of heat for sterilization, as high temperatures lead by killing the bacteria to denaturation of bacterial cell proteins.

Example: Coagulation of protein and egg while frying an egg. Preserving protein solutions on ice and avoiding high temperatures can limit temperature-caused denaturation. Higher temperature is a major stress for protein which produces damaged globular structure. A temperature plot is sigmoidal to the unfolded protein fraction, and the medium point (standing for the melting temperature) is indicated as Tm value. Increasing Tm value can assume the improvement in conformity stability. This could be exact in the thermal transfer to compare the reversibility degree between folded and unfolded form of protein.

Over the past 20 years, however, reversibility comparison has proved to be an even stronger indicator than Tm value for notice storage stability. Subsequently, other measures of conformation stability, such as those obtained from the studies of chemical denaturation, may be more efficient in decisions guiding on formulation. Using DSC, it is noted that denaturation induced thermally is an irreversible phenomena as molecules of unfolded protein easily combine and aggregate. Ever after the study by Sanchez-Ruiz et al. [81], there are frequent Tm′s rate dependence reports of DSC to examine rate of aggregation by adjusting the scan rate. More recent attempts to create more generalized kinetic schemes have been made. Mathematics assistance can be very much involved in overcoming some of the restrictions on the order of reaction of previous methods.

13.8.2 Freeze-Thaw

Freezing and thawing can interact with indigenous protein conformation and pH variability and buffer portion precipitation all of which can lead to protein denaturation. Freezing effects are determined by freezing to avoid the denaturation caused by freezing. Keeping aliquot of 50% glycerol so it would be easy to remove an

aliquot rather than freezing and deal with the whole solution. There are only few reports on cold denaturation of protein because this process has been recorded since 1961. Denaturation of protein is not of major significance because it is noted that majority of proteins denatured by cold only below than water freezing point. Tg′ is glass transition temperature of fully freeze state (< -20 °C). It means that proteins may have flexibility similar to that of a fluid solution in a frozen state of −20 °C. Cold denaturation potential may therefore be higher than what is expected. For instance, a recent IL-1ra study found that the temperature for cool denaturation is −10 °C and reachable easily in frozen condition except the temperature is < −30 °C in storage.

13.8.3 Physical Factors

Physical instability refers to the process by which by altering the chemical composition, the protein changes its physical structure. Owing to protein storage, physical denaturation happens, shaking triggers protein accumulation, vortex shaking, etc. Do not agitate, shake, or churn violently (don't foam protein solutions) and help prevent denaturation of protein. To avoid protein denaturation due to solution effects, replicate the cell environment and maintain ionic composition and neutral pH. Maintaining as much as possible protein concentration (> 1 mg / ml) dilution effects can be prevented.

13.8.4 Oxidation

Oxidation of proteins can disrupt protein stability and may use strong reducing agents such as DTT (or β-ME) in buffers to prevent oxidation-induced denaturation [15]. Proteins, often of major biological effect, have been known to be resistant to oxidize damage. Oxidized proteins have also been shown to increase in the aging of animals, thereby adding to the probability of protein oxidation leading to aging [33, 90]. Methionine has been shown to be oxidated into the form of sulfoxides in a broad variety of proteins and sometimes inhibits or prevents biological activities. As proteins are used as medicines, methionine oxidation is of particular concern [4]. Many atmospheric oxidants were also shown to produce the methionine sulfoxide in proteins and peptides [5, 58].

Oxidize methionine affects the organic role, in certain instances; it is known as destabilizer of native protein structure with apparent effects on activity [14]. It is well-known that substitution in the lateral chain – has major effects on stability. Further oxidation can be regarded as chemical mutagen, which substitutes the methionine side chain to methionine sulfoxide a larger mutated side chain. Surprisingly, the structure and stability of proteins would not be greatly affected. A potential way of protecting to methionine oxidation consequences, code a protein with other

residues of side chains which is known to resist the oxidation process. Altering active or binding sites is another attempt to avert the methionine oxidation which primarily leads to inactivity of proteins. Subtilisin, anti-trypsin, and D-amino acid oxidase are all oxidative inactivators that impact on methionine substitution [27, 79]. However, within just one case that we are aware of, the effect of methionine oxidation on stability combined with mutagenesis has been measured to anesthetize the protein toward oxidation. This distinction is significant; subsequently, a side chain substitution effects can be equally sensitive as this site is mutated by oxidation.

Eventually methionine oxidation can influence the protein structure and stabilization by reducing side chain hydrophobicity, increasing the hydrogen binding ability, and modifying methionine size and shape. Relative value of these variables depends on a given methionine's particular atmosphere. It can also be assumed that if the source of instability will change in side chain polarity after oxidation, then the results of nonpolar side chain substitution could be minimal. At oxidation of a given methionine residue, it is difficult to estimate the degree of stability changes, much less whether the side chain substitution is permanent.

13.8.5 Heavy Metals

Heavy metal salts typically contain Hg2 +, Pb2 +, Ag2 +, Ti1 +, Cd2 + and other weighed atomic metals. Since salts are ionic, salt connections break in proteins; the contact between heavy metal salts and protein usually leads to unsolvable metal protein salt. By reducing the denaturation of proteins, EDTA in buffers helps subsides heavy metals there.

13.8.6 Microbial Growth

Sanitized procedures that can include antimicrobials may be used to inhibit microbial production and/or frozen and to help prevent microbial growth.

13.8.7 Proteases

Proteases split the protein and thus absence the structure of the protein, leading to denatured proteins including protease inhibitors. Holding protein solutions on ice contributes to reducing protease effect.

13.8.8 Denaturation

Denaturation means that most proteins tolerate the loss of the globular or 3D shape. This globular configuration is called the native state, but it is well recognized to be merely a multiplicity of microstate. Denaturation can lead to loss of the secondary or tertiary structure (or both). Subsequently, it will denature the physical form, but chemical structure remains the same.

13.8.9 Chemical Denaturation

Chaotropes are the compounds that damage the globular structure of proteins by another technique of denaturation to unwind the protein; among these, guanidinium hydrochloride and urea are the most common. Urea also helps to prevent hydrophobic collapse linked to the development of a globular native structure. In comparison with absent solutes, chaotropes tend to bind proteins, which reduces their chemical potential. The protein unfolds to smaller than native state, so native state has usually much surface area than unfolded structure, and the chemical potency of unfolded state is further reduced. The pKa of polypeptide side chain has been reported to be modified by 0.3 to 0.5 units using high concentrations of GnHCI or urea. Through enhanced electrostatic repulsion, it alone can affect the protein's conformation stability.

13.8.10 Pressure-Induced Denaturation

The molecular ground for denaturing caused by pressure has recently been identified. Interestingly, the potential of osmolytes applied to stabilize proteins also tends to strain-caused denaturation. Furthermore, unlike other unfolding protein pressures, the pressure-induced denature is reversible. The intermediate pressures of 1000–1500 bar can be used to separate aggregates and enable the aggregated protein to be easily replunged.

13.8.11 Solid State Denaturation

As discussed above, at high temperature, proteins are denatured, but the mobility of proteins may presume in solid state. For dried proteins, the recorded denaturation temperatures are mostly very high, i.e., above 150 °C. Like Tg value, the Tm value seems to scale with humidity level. For example, for hGH, denaturation occurs just above Tg temperature, is largely irreversible and cooperative.

13.8.12 Intrinsically Denatured Proteins

There has been a discovery over the past decade that many random coiled proteins function as unfolded structure, under native conditions. These proteins are intrinsically denatured proteins (IDPs), whose certain pharmaceutical value proteins especially belong to superfamily of fibroblast growth factor. Proteins may also be interactive, without denaturation occurs in their globular structure, in the normal sense.

13.9 Limitations for Stability Studies

- Stability studies are valid only when the breakdown depends on temperature.
- Stability studies are accurate for only 10 to 30 kcal / mol activation energy. Most reactions have activation heat ~10 to 30 k cal/mole in solution phase. If the activation energy is <10 kcal / mol, its rate at room temperature would be rapid. Elevated temperature has slight effect on denaturation in these cases. If the activation energy is >30 kcal / mol, it needs very high temperatures for degradation. Reactions may be meaningless at such a high temperature, because they do not represent the state of ambient storage.
- The result reported for one set of formulation condition cannot be extended to another of the same product preparations.
- Stability prediction at high temperatures is of little benefit as diffusion, microbial contamination, and photochemical reaction result in degradation.
- Stability experiments are useless if the substance loses its physical integrity at higher temperatures such as suspending agent coagulation and protein denaturation.
- The prediction would be incorrect if the order changes at high temperatures, as in the case of a suspension (zero order) which at higher temperatures is converted to a solution following the first order.

13.10 Chemical Kinetics

It is the study of the rate of chemical processes or reactant transformations occurring in the products according to the basic mechanism, i.e., the mechanism of reaction.

13.10.1 History of Chemical Kinetics

By the implementation of law of motion and mass, Cato Guldberg and Peter Waage in 1864 stated the chemical kinetic studies. According to this, the speed of a reaction is directly proportional to available quality of reacting materials. Van't Hoff was awarded the First Nobel Prize in 1901 in chemistry. He published "Études de

dynamique chimique" and deliberated on chemical mechanics in gratitude of the strange amenities while researching on the law of osmotic strains and chemical kinetics. For zero-order reactions, simple rate laws exist, which require reaction rates to be taken irrespective of first-order reactions and the second-order reactions. Basic reactions obey the rule of mass movement, but the rule of the rate reactions step by step can lead to a mix of the rate laws from the different basic phases and can become very complicated. The first is van't Hoff wave, related to thermodynamic kinetics which is general chemical reaction laws. The second is focus on reaction mixture and called as Semenov Hinshelwood wave. Third wave is related entirely to mathematical science of chemical reaction networks.

13.10.2 Rate of Reaction

The sum of a chemical change occurring per unit time is called reaction rate. The rate is usually indicated as the increase or reduction in the concentration of a reactant or drugs, where the reactant concentration is dx / dt at any point [86].

13.10.3 Order of Reaction

The number of absorption conditions that depend on the speeds of the reaction is called reaction order. If reaction rate depends on the first reactant concentration, then the rate of reaction is = KC1. A chemical reaction whose intensity does not depend on the attention of the reactant is called a zero-order chemical reaction. So the reaction is presumed to be of first order. The second-order reaction presumed when the intensity is equal to the sum of absorptions of reactants or the square of absorption of a reactant.

13.10.4 Molecularity of Reaction

The molecularity of a reaction is known as the amount of molecules or atoms that create a part in a process of chemical change. According to one, two, or three molecules, the reaction is called unimolecular, bimolecular, and trimolecular. The term unimolecular was used in the first order for all reactions, the term bimolecular for second order reactions, etc.

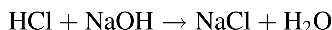
13.10.5 Types of Chemical Reactions

13.10.5.1 Instantaneous Reactions

Such reactions occur very quickly once the reactants are recognized. These reactions are called ionic reactions as they contain ionic compounds. It takes between 10–14

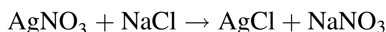
and 10–16 seconds for such reactions to initiate. Regulating the frequency of such reactions is typically unbelievable. There are two examples of this:

- When in aqueous solutions, an acid mixed with base, the reaction is called neutralization:



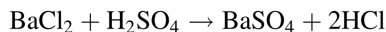
$$HCl + NaOH \rightarrow NaCl + H_2O$$

- When silver nitrate and sodium chloride solutions are combined, precipitation of AgCl occurs.



$$AgNO_3 + NaCl \rightarrow AgCl + NaNO_3$$

- By reaction of sulfuric acid and barium chloride, precipitation will occur [70].



$$BaCl_2 + H_2SO_4 \rightarrow BaSO_4 + 2HCl$$

13.10.5.2 Slow Reactions

There are very slow realistic reactions. It takes months to reveal any quantifiable change at room temperature. The kinetics of these reactions, too, are difficult to understand.

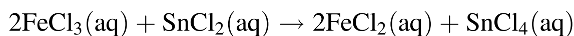
Some examples are:

- Hydrogen and oxygen reaction at room temperature. Atmospheric H2S clearly leads acetate reaction.
- Carbon and oxygen reaction: at 298 K, CO2 is more thermodynamically stable than carbon and oxygen, but coke remains unreacted for year and spontaneously does not catch fire in the soil.
- Carbon monoxide and hydrogen reaction: in actual practice, the reaction at 298 K bur is thermodynamically feasible [26].

13.10.5.3 Moderate Reactions

There are a number of reactions between the above two excesses that occur at room temperature at appropriate and evaluable rates, and in chemical kinetics, these reactions are studied. These reactions are normally molecular type. Below are some examples of this:

- Hydrogen peroxide decomposition: $2H_2O_2 \rightarrow 2HO + O_2$
- Decolorization of acidified potassium permanganate with sodium oxalate.
- Nitric oxide and chlorine reaction: $NO + Cl_2 \rightarrow NOCl_2$
- An ester hydrolysis: $CH_3COOC_2H_5 + NaOH \rightarrow CH_3COONa + C_2H_5OH$
- Nitrogen dioxide and carbon monoxide reaction. $NO_2 + CO \rightarrow NO + CO_2$
- Reaction between ferric chloride and stannous chloride.



$$2FeCl_3(aq) + SnCl_2(aq) \rightarrow 2FeCl_2(aq) + SnCl_4(aq)$$

- Cane sugar inversion in aqueous solution: $C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$
- Nitrogen pentoxide decomposition: $2N_2O_5 \rightarrow 2N_2O_4 + O_2$

Changing conditions under which they occur can decelerate or paced chemical reactions, e.g., the reaction is very slow. By maintaining temperature about 400oC and pressure about 300 atmospheres, CO + 2H2 CH3OH can be increased by using a ZnO and Cr2O3 containing catalyst. Decline in foodstuffs can be decelerated by refrigerating them [109].

13.10.5.4 Fast Reactions

For rapid reactions, the time needed may be similar or longer than reaction's half-life to combine the reactants and get them to a defined temperature. Specific solutions will minimize the time to the millisecond level for twitching rapid reactions. Flash photolysis, without a slow mixing step, include stopped flow techniques, where a laser pulse produces super excited speed.

13.11 Factors Affecting the Reaction Rate

13.11.1 Physical State

The physical state of a reactant (solid, liquescent, or vapor) is also a significant factor in the degree of alteration. Once reactants are as like as in aqueous solution, they may bring into contact by thermal movement. However, when they are in separate phases, the reaction to the interface between the reactants is incomplete. In the case of a liquid and a gas, reaction may only occur in interaction area. To complete the reaction, strong shaking and agitating may be appropriate. It means differentiation of the solid or liquid reactant, the greater the surface area per unit volume, and the greater the interaction with that other reactant, the more rapidly the reaction occurs. For example, you use wood chips and tiny branches to make a comparison, when you start a fire, you don't start with big logs right away.

13.11.2 Surface Area of Solid State

A reaction in a solid may involve only those particles that are on the surface. When a solid crushed into smaller particles, the reaction occurs more quickly. Because of the collision, probability between these smaller reactant particles was increased. For example, a mixture between a very fine weak organic acid malic acid and sodium hydrogen carbonate is sherbet (liquid).These chemicals dissolve and react rapidly in the mouth upon contact with the saliva, providing the taste of citrus and releasing

carbon dioxide. Fireworks manufacturers also regulate the rate at which the fireworks oxidize the fuels by adjusting the surface area of the solid reactants, to produce different effects. For example, finely split aluminum is exploding violently enclosed in a shell. The reaction is slower when larger aluminum parts are used, and sparks that is burning metal parts are shown as removed.

13.11.3 Concentration

The reactant species collide in these reactions. The frequency at which molecules or ions react depends on whether they occur faster is 21% O_2 (the pure oxygen). The more crowded these molecules are, the more likely they will collide with each other and react. As a result, a rise in concentrations of reactants will normally have an inverse effect usually consequence in decrease in concentrations and increase in rate of reaction. The rate equation explains the extensive reaction rate dependence on the reactants and other present species concentrations. The reaction mechanism depends upon the mathematical expression. For a given reaction, the actual rate equation is determined experimentally and describes the mechanism of reaction. The rate of mathematical equation is given by rate of constant reaction which is the molar concentration and partial order of reactant. A reactant's partial order could be experimentally calculated, and even its stoichiometric coefficient is not indicated.

13.11.4 Temperature

In general, temperature has a significant influence on the amount of a chemical reaction. They have higher temperatures and more thermal energy. While the incidence of collisions at higher temperatures is greater, this rise the reaction rates by a very small proportion. Most importantly, the proportion of reactant molecules with sufficient energy to react (energy higher than activating energy: $E > Ea$) is substantially higher and is explained in detail by molecular energy. A common misunderstanding is the "general rule" which doubles the rate of chemical reactions for every 10 ° C temperature rise. This may have been generalized from the biological systems' special case, where the A is often between 1.5 and 2.5. The method of temperature jumping may be used to study rapid reaction kinetics. This includes the use of a sudden temperature increase and measure of the time of stress relief to come back to equilibrium. A shock tube is a particularly useful form of temperature jumping system, which can quickly increase a gas's temperature by over 1000 degrees.

13.11.5 Catalysts

Generic energy potential diagram showing the catalyst effect in a hypothetical chemical endothermic reaction. The catalyst's presence opens a new reaction path

with lower energy activation. The end result is the same as for thermodynamics in general. A substance which is chemically unchanged but increases the chemical reaction is called catalyst. In lower activation energy, the catalyst increases the reaction rate by supplying a new mechanism for reaction to occur with. In a reaction, product itself is an autocatalysis with positive feedback. Enzymes are called proteins which function in biochemical reactions as catalysts. A catalyst does not affect the equilibrium path, because the catalyst accelerates the reactions backward and forward equally. Related substituents in some organic molecules may influence the rate of reaction in the involvement of neighboring group.

13.11.6 Pressure

The number of reactant collides, and the rate of reaction increases by increasing the pressure in a gas reaction. This is because a gas's activity is directly proportional to the gas's component pressure. That is similar to the product of a solution's rising concentration. Beyond clear effect of mass action, the coefficients of the rate itself change because of strain. The rate coefficients and products of such high-temperature gas-phase reactions alter when an inert gas is added to the mixture; changes to this effect are called fall-off and chemical activation. Such results are due to exothermic or endothermic reactions occurring faster than heat transfer, causing nonthermal distributions of energy for the reacting molecules. By increasing pressure, the rate of heat transfer between reacting molecules and the rest of system increases. Thus, pressure can also affect the coefficients of the condensed-phase scale by reducing this effect, but a measurable effect requires very high pressure because the ions and molecules are not very compressible. This effect is studied also using diamond anvils. The kinetics of a reaction can be studied with a pressure jumping method which includes time recovery and quick adjustment in pressure to be back to normal.

13.11.7 Absorption of Light

Study of light-initiated reactions with photosynthesis being one of the prominent examples of photochemistry. When one reactant is pushed to an excited state by absorbing an appropriate wavelength of light, the activation energy can be provided to it.

13.11.8 Free Energy

Generally speaking, the free energy transfer of a reaction determines that there is going to be a chemical shift; however, kinetics describes how quick the reaction is. A reaction may be very exothermic and can result in a very positive increase in entropy, but in reality, it will not occur if the reaction is too late. If a reactant may produce two

products, it typically forms the most thermodynamically stable one, except in particular circumstances where it is stated that the reaction is under kinetic reaction. Predictions can be produced for a reaction from free energy relationships on constants in reaction rate. The kinetic isotope effect is the difference in the rate of a chemical reaction when one of the isotopes in one of the reactants replaces an atom. Awareness of heat transfer and residence time in chemical engineering in a molar mass distribution and nuclear reactor in polymer chemistry are given by chemical kinetics. It also includes the knowledge of corrosion engineering [70].

13.11.9 Stability Testing

A drug product can undergo changes in consistency, appearance, uniformity, clearness (solution), moisture content, package integrity, pH, particle size, and shape all affecting its stability. These physical changes can occur due to variations in impact, vibration, abrasion, and temperature such as freezing, thawing, shearing, etc. The chemical reactions that occur in pharmaceutical products such as solvolysis, oxidation, reduction, racemisation, etc. can lead to degradation of drug formation, loss of active pharmaceutical ingredient potency (API), loss of excipient activity such as antimicrobial preservative action and antioxidants, etc. [9]. Microbiological development such as non-sterile microorganism and improvement of preservative performance affected the pharmaceutical product stability [65]. Table 13.2 illustrated possible adverse effects of volatility on pharmaceutical products.

13.12 Need and Purpose of Guidelines for Stability Studies

In order to ensure that good drugs are developed that may be sufficiently potent to last until their duration of stability is well promoted and reaches people on time. In many countries, authorities have concerned that the manufacturers should make available information about the power or consistency of the same drug or the length of its shelf life. The goal was to bring all manufacturers to similar test methods. The guidance embodies the simplest drug / stability-related problems. It includes documentation on the way to qualify for drug manufacture by supplying requisite details on shelf life, efficacy, and durability of a drug and strategies for applying it. Such kinds of recommendations emerged first in the 1980s (www.ICH.org).

13.12.1 Brief History

In 1987, the FDA provided its initial guidelines (Huynh 2010).

Table 13.2 Potential adverse effects of instability in pharmaceutical products

Potential adverse	Potential adverse	Potential adverse	Potential adverse
Loss of active ingredient	Degradation of API in product resulting in less than 90% drug as claimed on label – unacceptable quality	Nitroglycerine tablets	Time elapsed before the drug content no longer exceeds 90%
Increase in concentration of active ingredient	Loss of vehicle perfusion bags sometimes Allow solvent to escape and evaporate so That the product within the bag shows an increase in concentration.	Lidocaine gel, products in perfusion bags	Stability in final container
Alteration in bioavailability	Changes in rate and extent of absorption on storage	_	Dissolution/ release studies
Loss of content uniformity	Loss of contents as a function of time	Suspension	Ease of re-dispersion or sedimentation volume
Decline of microbiological status	Increase in number of viable microorganisms already present in the Product. Contamination because of compromised package integrity during Distribution/ storage	Multiuse cream	Total bioburden after storage
Loss of pharmaceutical elegance and patient acceptability	Speckling caused by the interaction of the drug containing amine group with a minor component in the lactose resulting in the formation of a chromatophore	Slight yellow or brown speckling on the surface of tablet containing spray dried lactose	Visual examination
Formation of toxic degradation products	Degradation of the drug component	Formation of epianhydrotetracycline from tetracycline, protein drugs	Amount of degradation products during shelf life
Loss of package integrity	Change in package integrity during storage or distribution	Plastic screw cap losing back-off-torque	Specific package integrity tests
Reduction of label quality	Deterioration of label with time and cause the ink to run and thus adversely affect legibility	Plasticizer from plastic bottle migrates into the label	Visual examination of the label
Modification of any factor of functional relevance	Time-dependent change of any functionally relevant attribute of a drug product that adversely affects safety, efficacy, or patient acceptability or ease of use	Adhesion aging of transdermal patches	Monitoring changes

13.12.2 Guidelines by the Food and Drug Administration

- Incorporate medication safety designs, correct expiry dates, storage methods, and precautions to be taken during medication storage.
- To apply data on the investigational stability analysis of synthetic products, biologics, experimental drug applications, and the application for a biological product license have stressed upon.

Later, various regulatory bodies from different countries established their own strategies. Because of discrepancies on these guidelines, the need to harmonize the guidelines was felt strongly. In the International Conference on Harmonization (ICH), regions, such as the USA, Japan, and Europe in the 1990s, make efforts in stability practice to bring uniformity. They made uniform products data in the ICH at a later date to register and promote in various regions. The ICH was a union where the regulatory and manufacturing industries of ICH regions made daily suggestions. It also extended earlier ICH guidelines for veterinary products [52].

13.12.3 Benefits of Regulatory Harmonization

For each changing regulatory authority, regulatory harmonization provides many direct advantages, as well as the manufacturing trade with useful advantages required to protect people's health. The key benefits are preventing the duplication of human clinical trials, thus reducing animal testing and without sidelining shelter and efficiency, controlling, formulating the procedure for testing new applications of drugs, and reducing development period and cost of drug production [52]. Slow but great developments have taken place in the International Conference on Harmonization since its participation in 1990, and the ICH has seen significant growth in its initial period of entry into action, in particular in the areas of health, safety, and effectiveness. There have been studies on multidisciplinary topics, including Common Technical Document (CTD) and Medical Dictionary for Regulatory Studies (MedDRA) [11, 89].

13.12.4 ICH Guidelines

In November 2005, the International Conference on the steering committee for harmonization assigned codification for ICH Guidance. The goal of allocating new codes was to make it easier to enforce in practice, and sure there is ambiguity in it. The allocated codes are based on a number of revisions such as (R1), (R2), and (R3). This was done to make guideline codification for ICH easier for all. Several secessions have also been applied to the guidelines, and the key or core guidelines are called revisions (e.g., R1) [11, 89].

13.12.5 General Categories of ICH Guidance

The following groups and codes were assigned according to www. ICH.org.

13.12.6 "Q" Guidance

Efficiency guidance within the main area of harmonization encompasses critical achievements, as do methods of understanding drug stability studies, setting minimum thresholds needed to check impurities in the studies' products and to determine the quality of the goods produced in accordance with good manufacturing practice (GMP) risk management [89]. The ICH-released "Q" guidelines are listed in Table 13.3.

13.12.7 "S" Guidance

These are health recommendations that provide safety precautions to reject serious problems such as gene toxicity, cancer, and kidney toxicity. The ICH-issued "S" recommendations are specified in Table 13.4.

13.12.8 "E" Guidance

These guidelines regulating the manner of planning trials, trails performing, and the shelter measures taken and reporting on the clinical trials that have been carried out. This also regulates the various vital forms of medicinal products which are attained by the adoption of specific biotechnological procedures. Additionally, the use of pharmacokinetics and pharmacogenomics methods are applied in the development of the best medicines. The ICH-released "E" Guidelines are listed in Table 13.5.

Table 13.3 Showing ICH "Q" guidelines. Source: www.ICH.org

Q1 A –Q1 F	Stability guidelines
Q2	Analytical validation
Q3 A –Q3 D	Impurities
Q4 –Q4 B	Pharmacopoeias
Q5 A –Q5 E	Quality of biotechnological products
Q6 A –Q6 B	Specifications
Q7	Good manufacturing practices
Q8	Pharmaceutical development
Q9	Quality risk management
Q10	Pharmaceutical quality system
Q11	Development and manufacture of drug substances
Q12	Lifestyle management

Table 13.4 Showing ICH "S" guidelines. Source: www.ICH.org

S1 A –S1 C	Carcinogenicity studies
S2	Genotoxicity studies
S3 A –S3 B	Toxicokinetic and pharmacokinetics
S4	Toxicity testing
S5	Reproductive toxicology
S6	Biotechnological products
S7 A –S7 B	Pharmacology studies
S8	Immunotoxicology studies
S9	Nonclinical evaluation for anticancer pharmaceuticals
S10	Photosafety evaluation
S11	Nonclinical safety testing

Table 13.5 Showing ICH "E" Guidelines. Source: www.ICH.org

E1	Clinical safety for drugs used in long-term treatment
E2 A-E2 F	Pharmacovigilance
E3	Clinical study reports
E4	Dose response studies
E5	Ethnic factors
E6	Good clinical practice
E7	Clinical trials in geriatric population
E8	General considerations for clinical trials
E9	Statistical principals of clinical trials
E10	Choice of control group for clinical trials
E11	Clinical trials in pediatric population
E12	Clinical evaluation by therapeutic category
E14	Clinical evaluation
E15	Definitions in pharmacogenetics and pharmacogenomics
E16	Qualifications for genomic biomarkers
E17	Multiregional clinical trials
E18	Genomic sampling methodologies

13.12.9 "M" Guidance

These are multidisciplinary guidelines, in which cross-cutting disputes do not activate clearly within the reference classes of health and effectiveness community. It includes the International Conference on the Common Technical Manual (CTD) and Medical Terms of Harmonization (MedDRA) and the establishment of Electronic Standards for the Transmission of Regulatory Knowledge (ESTRI). Table 13.6 lists the "M" Guidelines established by the ICH.

Table 13.6 Showing ICH "M" Guidelines. Source: www.ICH.org

M1	MedDRA terminologies
M2	Electronics standards
M3	Nonclinical safety studies
M4	Common technical document
M5	Data elements and standards for drug dictionaries
M6	Gene therapy
M7	Genotoxic impurities
M8	Electronic common technical document (eCTD)

13.13 Guidelines for Stability Studies

13.13.1 ICH and FDA Stability Regulatory Guidelines

One of the subject areas protected by the guideline documents of the ICH is checking stability for drug registration. The ICH mutually administers managers and pharmaceutical companies from the USA and Japan converging all the technological measures for drug-containing medical products. In the early 1990s, this organization was launched, and one of the first topics was stability monitoring to be carried out by regulatory bodies from all three regions through the stepwise method of recognition [19].

Currently, with in manufacturing industries, stability studies are a significant approach approved for emerging brand new drugs and products. Stability analysis is also used to recommend desirable situations for storage of the imports and emphasize the fact for marketing purposes the expiry date and potency details of the drug must be specified on the cover of the outer packaging of the product. Specifying that the drug is harmless and effective up to its expiry date definite on suggested storage conditions is added to the outer packaged of the product. It is certified on the label inside that the medication is harmless and operative for its entire shelf life. Monitoring requirements being made tighter and tighter to touch the optimum target of each possible situation so that the medication can be put during its shelf life. Therefore, after the adoption of good science principles, the stability studies can be performed by accurately understanding the standing regulations regulating the same and also taking into account the environment sectors [89].

13.13.2 Guidelines

To precisely illuminate the details of stability required to record the new drug and products, a series of guidance documents were created within the ICH regions. These guidance documents will adhere the stability practice conducted for registration of products. There are currently five online guidance documents.

13.13.3 Q1A

Stability testing of new drug materials and goods lays down the basic procedure for registration stability studies (www. ICH.org). In both materials and goods of new drug, this monitor sets out types and number of lots, stabilization vessel closing schemes, and time arguments, and storage situations are revised to endorse registry. The ICH guidance documents reference for more comprehensive details on specifications and impurities. Correct measurements, diagnostic procedures, and proposed acceptance requirements should be used. ICH document reports pressure testing and essential assertions to offer details on new drug material and its stability to three product lots at least, during the recommended period of expiry or retesting [47].

13.13.4 Q1B

The four remaining documents are general guidelines for the protocol. There is the Q1B guideline and photostability checking for new drug drugs and products (www. ICH.org). This article provides recommendations for photostability research on new drugs and drug products that demonstrate that light exposure does not impact materials adversely. The work outlined is performed in a single batch from the stabilization analysis of registration and constitutes a step-by-step process involving exposed medication content and exposed formulation of the drug, shortly following packaging and packaging for distribution product formulation [45].

13.13.5 Q1C

Guidance Q1C provides information about testing stability of new dosage (www. ICH.org) was written by the holder of the initial submission to explain the criteria for a new dosage form or line extension. In this case, Q1A criteria were followed, but at the time of submission, less data may be needed. In the Q1A parent guidance, the use of brackets or matrix is stated to minimize the amount of testing for registration associated with the stability program.

13.13.6 Q1D

Guidance Q1D offers more comprehensive matrixing and bracketing proposals for drug substance and drug product stability testing [46] on the subject. The guide addresses when to use each of these methods and offers examples of them. This also addresses the short prototype possible dangers for testing.

13.13.7 Q1E

The fifth guidance, Q1E, Stability Data Assessment [47], offers additional details regarding the method of assessing and analyzing the produced details, statistically following the Q1A guideline. This guide includes a step-by-step method for evaluating the data on stability and extrapolating the knowledge obtained to indicate the product's expiry date. It addresses applying linear regression, pool capacity checks, and statistical modeling to registration stability results.

13.13.8 Q5C

An additional guidance has been drawn up to complement these guidance documents for the study of biotechnology products [44]. The guidelines set out only key stability registration system requirements. All the information required for designing and maintaining the stability system to facilitate the registration of new goods. In addition, the brief applications for the registration of generic medicinal products go beyond the scope of the ICH documents, but the guidance documents may be supplemented by general guidelines in order to facilitate registration studies. A paper published by the FDA in 1987 offers further guidance and recommendations for sending reports on the stability of human drugs and biologics [28]. In 1998, the FDA developed a draft guideline for industry following this paper: stability testing of drug substances and medicinal products [29].

13.13.9 ICH Q1AR2

It merged ICH Q1AR2 with several different guidelines from the ICH guidelines. For those who are carrying out stability studies, this guideline acts as basic guide. In the year 2004, the International Conference on Harmonization provided Q1F guidelines, which recommended ongoing stability research programs to facilitate third and fourth zones. The Association of Southeast Asian Nations (ASEAN) pronounced the requirements needed to be met and enforced for the extremely hot and humid climate.

In June 2006, both documents of the FDA, one is the stability guidance and the other is the draft guidance, were removed. As a result, in July 2006, the ICH revoked the ICH Q1F guideline. As part of the agency's implementation, sustainable production practices in pharmaceutical current (cGMPs) for the twenty-first century emerged ([30]; www.fda.gov/cder/gmp).

The QbD, quality by design principles established by the Food and Drug Administration in drug development, has been the most debated subject of all time. For easy reference, Table 13.7 shown the ICH assigned names and their respective codes of Harmonization guidelines (www.ICH.org; [51]). In addition to different factors that influenced the stability, the guidelines took account of the climatic regions. Specific

Table 13.7 Codes and titles used in ICH Guideline. Source: www.ICH.org

Q1A	Stability testing of new drug substances and products (second revision)
Q1A (R2) 2	Stability testing of new drug substances and products
Q1B	Stability testing: Photo stability testing of new drug substances and products
Q1C	Stability testing of new dosage forms
Q1D	Bracketing and matrixing designs for stability testing of drug substances and products
Q1E	Evaluation of stability data
Q1F	Stability data package for registration applications in climatic zones III and IV
Q5C	Stability testing of biotechnological/biological products

guidelines for the particular drug delivery system were drafted for each product. In addition, guidelines were also developed from the accelerated studies for inferences.

13.14 CPMP Stability Guidelines

Under the European Agency for Assessment of Medicinal Products, the Committee on Planted Medicinal Products (CPMP) published a set of guidelines relating to stability studies in contribution of seeking market authorization for medicines beyond their acceptance of the ICH instructions [19, 52].

These guidance documents are as follows:

13.14.1 Guidance CPMP/QWP/122/02

Checking stability of known active substances and associated finished products [21].

13.14.2 Guidance CPMP/QWP/576/96

Stability testing for applications for marketing authorization variations [20] offers the information required to perform studies on product stability that should be produced in support of variations made to marketing authorization. It gives some examples of variations and the stability data types and quantities that can be required to sustain them.

13.14.3 Guidance CPMP/QWP/2934/99

In-use stability testing of human medicinal products [23] offers the requisite details required to perform stability studies to assess the length of time a multidose product should be used after it has been first opened.

13.14.4 Guidance CPMP/QWP/159/96

Maximum shelf life for human use sterile products after first opening or subsequent reconstitution [22] notes that research should be carried out to confirm the realistic usage of sterile product. It also includes sample wording to provide the correct hold times and storage conditions in consumer labeling until the product is opened, diluted, or reconstituted.

13.14.5 Guidance CPMP/QWP/609/96

Accordingly, the declaration of storage conditions has been defined as follows: A: in the stock details of medicinal products and B: for active substances [25], document providing standard commodity storage status declaration shall contain more details (Annex) to the ICH Stability Guidelines. On the basis of the stability data generated according to the guidelines given by the ICH, an appropriate storage labeling statement and, if applicable, further storage statements are recommended.

13.14.6 Guidance CPMP/QWP/072/96

The beginning of a pharmacological shelf life [24] explains how a pharmaceutical product's expiry date can be calculated and assigned depending on the period of release or manufacture. For easy reference, the guidelines are mentioned in Table 13.8.

Table 13.8 Codes and titles used in ICH guidelines. (CPMP/QWP/122/02 2003)

CPMP/QWP/576/96 Rev. 1	Guideline on stability testing for applications for variations to a marketing authorization
CPMP/QWP/6142/03	Guideline on stability testing for active substances and medicinal products manufactured in climatic zones III and IV to be marketed in the EU
CPMP/QWP/609/96 rev. 1	Note for guidance on declaration of storage conditions for medicinal products particulars and active substances
CPMP/QWP/122/02 rev. 1	Note for guidance on stability testing of existing active substances and related finished products
CPMP/QWP/072/96	Note for guidance on start of shelf life of the finished dosage form
CPMP/QWP/2934/99	Note for guidance for in-use stability testing of human medicinal products
CPMP/QWP/576/96	Note for guidance on stability testing for a type 2 variation to a marketing authorization
CPMP/QWP/159/96	Note for guidance on maximum shelf-life for sterile products after first opening or following reconstitution

13.15 WHO Stability Guidelines

The World Health Organization (WHO) has provided guidelines on stabilization research [19]. The research on stability studies started in the year 1988 by the World Health Organization. In the technical expert, list No. 863 of Annex-V of the WHO was given guidelines for stability studies of drug substances in traditional dosage to prepare pharmaceutical requirements to expert committee [106]. Refusal to follow ICH guidelines to improve extreme weather conditions in many countries and announce only new medicines and products and no medicine recommendations already available on the markets of World Health Organization countries [52]. In the year 1996, the World Health Organization made some amendments to the International Conference on Harmonization. This advice was adopted in 2003 and 2006 to benefit for zone IV climatic areas for owing long-term storage conditions [107, 108]. In the year 2004, the World Health Organization also released guidelines on stability checking in the global environment [52].

The new World Health Organization's first draft, Stability Guidelines, were published in the year, April 2007, for feedback and suggestions. In October 2007, on the basis of the guidelines for stability of the eastern Mediterranean region of the WHO, the second draft was available. In addition, technical monograph of testing stability of new drugs and products has been published by Indian Drug Manufacturer's Association. In addition, other checking criteria for drug formulation and active pharmaceutical substance have also been set out in the guideline [52]. As other not specifically mentioned countries, many have accepted the ICH or WHO criteria for their stability assessment criteria [19].

13.16 Stability of Climatic Zones

Based on the environmental conditions, the entire planet has been divided into four zones to which pharmaceutical products for stability purpose are likely to be exposing in their storage period.

For these areas, these parameters were measured on the basis of mean annual temperature and relative humidity. On the basis of that data, long-term or real-time stability test conditions and accelerated stability test conditions have been established. The traditional climate zones for use in stabilization studies of pharmaceutical products were presented in Table 13.9. The rise up of ambient conditions in each zone and the resulting long-term stability test storage conditions as given by the WHO were also presented. The stability needs have also been harmonized to make the implementation of industry more practical and reliable for widespread use [48].

13.16.1 Mean Kinetic Temperature

A single temperature at which the total degradation in a given time is equal to cumulative degradation of individual degradations occurring in a different higher or

Table 13.9 ICH climatic zones and long-term stability conditions. [37, 48]

Climatic zone	Climate/ definition	Major countries/ region	MAT[a]/Mean annual partial water vapor pressure	Long-term testing conditions
I	Temperate	United Kingdom, Northern Europe, Russia, United States	<15 °C/<11 hPa	21 °C/45% RH
II	Subtropical and Mediterranean	Japan, Southern Europe	>15–22 °C />11–18 hPa	25 °C/60% RH
III	Hot and dry	Iraq, India	>22 °C/<15 hPa	30 °C/35% RH
Iva	Hot and humid	Iran, Egypt	>22 °C/>15–27 hPa	30 °C/65% RH
IVb	Hot and very humid	Brazil, Singapore	>22 °C/>27 hPa	30 °C/75% RH

[a]MAT – Mean annual temperature measured in open air

lower time span is mean kinetic temperature (MKT). MKT is an isothermal storage temperature which simulates the non-isothermal effects of variations in storage temperature. MKT takes frequent and seasonal in temperature over a year. It reflects the accumulated thermal stress a commodity undergoes during storage and delivery at various temperatures. The definition of MKT is applied to ensure that the real storage conditions do not negatively impact the product's shelf life and stability. It focuses on the fact that the degradation rate constants are temperature dependent. Thermostatically adjusted room temperature to the usual operating range of 20 °C–25 °C results in a kinetic average temperature to not exceed 25 ° C. It includes stores, clinics, dealers, and storage areas, as well as vehicles and warehouses. Articles can be defined either by the term "restricted room temperature" or by a term/phrase of some other suitable definition based on the same kinetic temperature. The distribution in four different climatic zones of world countries and regions was focused on mean cinematic temperatures ([38, 48]; USP 1995). To calculate a mean kinetic temperature, i.e., two processes are used; USP program and FDA process MKT in the USP cycle are based on average storage temperatures over the course of 1 year, while the actual working weekly average for the preceding 52 weeks is high and low. This resulted in 52 data points, and the Hayne equation is calculated, which derives from the Arrhenius equation and relates the deterioration rate constants to activation energy at different temperatures.

$$MKT = \frac{\frac{-\Delta H}{R}}{ln\left(\frac{e^{-\Delta H/RT1}+e^{-\Delta H/RT2}+\ldots+e^{-\Delta H/RT_n}}{n}\right)}$$

Where the MKT = mean kinetic temperature; R = universal constant of gas, i.e., 83.14 kJ/mol;

ΔH = the energy of activation; T_1 = arithmetic mean of lowest and highest temperature recorded during first week; T_2 = arithmetic mean of lowest and highest temperature recorded during second week; n being the total number of average storage temperatures recorded during the annual observation period; and all temperatures T being absolute temperatures in degrees Kelvin (K). MKT calculating approach by entering the actual temperature values (rather than average values) is an equation proposed by the FDA. This result in oppose of point 52 and in favor of 104 points of USP. If temperatures are electronically registered during a day in several times, then there is no difference between the FDA and USP system.

13.17 Importance of Protein Stability

The main reason for the stability test is the interest of the patient with the condition for which the medications are intended. In addition to itemization of the unstable drug into toxic decomposition products, loss of operation up to the label stage of 85% may lead to therapy failure resulting in deaths such as angina and cardiac arrests by nitroglycerine tablets. Because of this issue, the supply of data for some forms of stability checks to regulatory agencies until approval of a new product has become a legal necessity. The second important issue is to maintain the manufacturer's image by ensuring that the product is kept fit for use in relation to all technically related features as long as they are on the market. Some advantages of stability studies at the manufacturing stage or of the products being sold are to provide a database of interest in the collection of suitable ingredients, excipients, and container closure systems for the production of a new product; to assess the shelf life and storage conditions for the production of a new product; to prepare the registration dossier; to substantiate the claimed shelf life for the registration dossier; and to verify that no changes have been made to the formulation or manufacturing process which could adversely affect the product's stability [9, 87].

The field of protein stability made groundbreaking progress in the last 20 years. Chemical instability pathways can now be better understood for better adjustment of the conditions of the solution. It in turn leads to reducing chemical degradation. Instability of proteins can be divided into two main groups, namely, physical instability and chemical instability. The word protein physical instability involves modification of the protein's physical state by precipitation (insolubility), aggregation, adsorption, and denaturation. Chemical instability involves certain processes that break or render covalent bonding, resulting in the creation of new, novel chemical entities.

13.17.1 Chemical Instability

Deamidation is a very popular chemical degradation pathway for proteins and peptides. This process includes the hydrolysis of Gln and Asn amides side chain [18]. Deamidation of Asn takes place under acidic conditions via direct hydrolysis of Asn side chain to make Asp. So the deamidation undergoes acid catalysis [34]. This mechanism occurs at pH 3 and does not involve cyclic imide formation at all [95]. Deamidation of Gln residue is far less common than Asn residues and is generally seen in larger proteins like monoclonal antibodies (Mabs) and crystallins. Cyclization method involves in Gln residues and generates a six-membered ring structure and is considered as less thermodynamically stable than Asn residual five-membered ring structure [83].

13.17.2 Physical Instability

Physical instability refers to the change in physical state of proteins without any change in its chemical composition. Some important physical instability methods are described below.

13.17.3 Denaturation

An important physical instability method is denaturation, which is basically the loss of three dimensional or globular structure of protein. It may also include the loss of secondary and/or tertiary structure of proteins, or may be both [41]. Denaturation may be thermal, cold, chemical, and pressure induced. Thermal denaturation means distortion of globular structure of proteins by elevated temperature. In general, an increase in temperature results in an increase in conformational stability, and also thermal denaturation is irreversible, as the protein structure rapidly changes from folded to unfolded ones, resulting aggregate formation [69].

Cold denaturation refers to the denaturation of proteins by employing "free concentrated states." In this regard, the recommended cold denaturation temperature is approximately 10 °C [94]. Another method of denaturation of proteins is chemical denaturation, which involves the unfolding of globular structure of protein by adding some chemical compounds like chaotropes, urea, and guanidinium hydrochloride (GnHCl) [72]. The mechanism by which these compounds destabilize or distort the globular structure is still not known very well [1]. Pressure-induced denaturation is a reversible process, involving the unfolding of proteins by applying pressure greater than 2000–4000 bar (atmosphere).

13.17.4 Aggregation

Protein aggregation is one of the most cited challenges in the development and manufacturing of protein therapeutics. The term protein aggregation refers to the many types of molecular assemblies. Aggregation is a reversible process and occurs either from covalently or non-covalently linked species [101]. Some causes of protein aggregation are misfolding during protein expression, freeze-drying, freeze-thawing, perturbation during protein purification, ultrafiltration, syringe filling, transportation, diafiltration, storage, etc. [62, 63]. All of these processes affect the product stability by directly exposing proteins to damaging/adverse conditions like extreme non-native protein aggregation has gained much attention because of increased adverse immunogenic effects during therapy, reduced efficacy of biological molecules, and turbidity caused by aggregated proteins and hence reducing the pharmaceutical elegance.

Despite the great potential of protein aggregation, it is very important to control protein aggregation during storage and processing. In this regard, protein-protein interaction and intrinsic conformational stability of protein play a key influential factor in modulating protein aggregation [54].

13.17.5 Precipitation

Precipitation basically refers to particle or particulate formation. This is a reversible behavior, in which proteins are either partially or completely unfolded. Protein solubility serves as an important factor in precipitation of proteins and is completely reversible upon dilution [64].

13.17.6 Surface Adsorption

Since the protein encounters many surface interactions during bioprocessing, interfacial stability is considered as a key factor. As adsorption itself is considered as physical instability, interfacial stress is even more problematic. Thus, interfacial stability greatly depends on several factors like property of protein molecule, available surface area, surface tension, and structural stability. Proteins are adsorb to various surfaces in aqueous form as well as in partially unfolded form due to increased exposure of hydrophobic amino acid chains [98].

13.17.7 Improving Protein Stability

Several protein stabilization methods have been well identified. Some of which are chemical modification, drying including lyophilization, stabilization by ions, and site-directed mutagenesis. By chemically modifying proteins with glycosylation, post-translational modifications may provide some means of altering physical and

chemical stability of a protein [77]. Among synthetic processes, PEGylation (addition of polyethylene glycol PEG) is very well-known to modify or alter the protein and also to improve physical and conformational stability of a protein [99].

Another method for improving protein stability is freeze-drying, which provide advantages of long-term storage stability, improved shipping, and also improved stability to varying degrees of temperature. Drying methods may also include spray drying, air drying, vacuum drying, film drying, and supercritical fluid drying [51]. Another important and well-known method to improve the solubility, chemical stability, as well as physical stability of proteins is "site-directed mutagenesis" which is a specific method to create targeted changes in double-stranded plasmid DNA [32].

13.18 Chemical Kinetics of Proteinous Compounds

Chemical kinetics basically deals with the rates of chemical processes. A chemical process is usually broken into a series of steps known as elementary reactions. These elementary processes involve a transitional state between two molecular and atomic states through a separated potential barrier. This potential barrier consists of activation energy of the process and is also responsible for determining the rate of reaction. This potential barrier is also an important source of temperature dependence of the reaction rate. Chemical kinetics of a reaction is usually performed to find out the reaction mechanism of a proteinous compound and also to determine absolute rate of reaction and individual elementary steps involved in proteinous compounds [97].

13.19 Applications and Advantages of Stability Studies

Chemical kinetics of protein compounds provide chemical engineers and chemists with different methods and tools to better describe the chemical processes like microbial growth, chemistry of biological systems, food decomposition, and stratospheric decomposition. Chemical kinetics can also be used to design and modify chemical reactors to optimize pharmaceutical product yields and also to eliminate environmental dangerous by-products. Kinetic methods may also be used to calculate temperature and pressure ranges for the maximum yield of hydrocarbons into gasoline.

There are several advantages of using chemical kinetic methods of proteinous compounds. Firstly, we can easily identify the transition intermediates and their concentration rates. In addition, they also allow large number of chemical reactions that can be easily used for analytical purposes. We can easily determine factors affecting rate of reaction, and the mechanism of chemical reaction can also be easily determined by using chemical kinetic methods of proteinous compounds [88].

13.20 Role of Pharmacists in Protein Stability

It is very necessary for a pharmacist or a pharmaceutical scientist to understand, to study, as well as to interpret the conditions of instability of any pharmaceutical product and also able to offer possible solutions to stabilize these pharmaceutical products. It is also the responsibility of a pharmacist to properly define the reaction rate, molecularity, and reaction order while applying zero order kinetics to the routine practice of pharmacy. In addition, a pharmacist should also be able to calculate shelf life and half-life of any pharmaceutical drug or product. It is very critical to interpret pH profile rates, kinetic data. So, maintenance of stability testing protocols, stabilization techniques, and regulatory requirements should be updated by a pharmacist. Therefore, stabilization of pharmaceutical agents is very essential to make acceptable products in community and the industrial pharmaceutical setting.

13.21 Conclusion

Stability testing is now the key procedural components in the pharmaceutical development for a new drug as well as new formulation. Stability test are carried out so that recommended storage conditions and shelf life can be included on the label to ensure that the medicine is safe and effective throughout its shelf life. There are several advantages of using chemical kinetic methods of proteinous compounds. We can easily identify the transition intermediates and their concentration rates. Thermodynamics also establish the necessary conditions for crystallization, and the kinetics dynamics of the processes determine whether a possible scenario actually becomes reality. The present study sums up the important land marks in the development of the guideline for stability studies. It is hoped that a ready to start reference is generated by the FDA, ICH, WHO, and CPMP guidelines to specific conditions. Therefore, stability tests should be carried out by following proper scientific principles and understanding of the current regulatory requirements as per the climatic zone.

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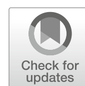

Stability Studies of Extemporaneous Pharmaceutical Products

14

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Abstract

Extemporaneous pharmaceutical preparations are the products that are compounded for an individual patient for a specific condition. Such preparations are usually compounded and dispensed due to lack of suitable dosage form, medicine dilution for pediatrics and geriatrics, lack of commercially available topical preparation, or absence of license medicine for certain diseases. Therefore, the extemporaneous pharmaceutical products must abide by the standards of compounding to ensure the safety and efficacy of the drug. The extemporaneous preparations fall under two broad categories of simple and complex ones. Simple extemporaneous preparations are the preparations that do not require any specialized facilities such as topic creams, ointments, tablets, and suppositories, whereas the complex extemporaneous parenteral and ophthalmic preparations require specialized equipment and training. The oral liquid dosage forms are most commonly prepared extemporaneously among all these preparations. The stability of extemporaneous preparations is highly critical; hence the formula utilized for extemporaneous dispensing should be preferably validated and have sufficient stability evidence. Each extemporaneously prepared dosage form possesses distinct stability testing and must be evaluated against these parameters. In general,

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_14

good laboratory practices must be satisfied while compounding extemporaneous preparation. The stability studies of extemporaneous preparations along with suitable examples are briefly discussed under this chapter.

Keywords

Extemporaneous preparations · Drug stability

14.1 Extemporaneous Pharmaceutical Products

An extemporaneous pharmaceutical product is a preparation specifically compounded to cater the needs of an individual patient [1]. A number of licensed medicines are dispensed by the pharmacist for safe and effective treatment. However, there are few pathological conditions in which no licensed medicine is available to fulfill the clinical needs of the patient. Under such circumstances, it is indispensable for the pharmacist to extemporaneously prepare a small amount of pharmaceutical product for an individual patient [2]. Similarly, lack of drug or a suitable dosage form can also be resolved by readily compounding an extemporaneous preparation for the patient. Compounding is defined as the preparation of a limited quantity of product only for the immediate use by the specified consumer. It is also called as extemporaneous dispensing. It involves preparing a pharmaceutical product directly from the raw materials or through modification of already manufactured products [3]. According to the United States Pharmacopeia, compounding or extemporaneous dispensing is defined as the preparation, mixing, assembling, packaging, and labeling of a drug in order to fill the prescription based on the practitioner/patient/pharmacist/compounder relationship in the course of professional practice [4]. Oral liquid dosage forms are most commonly prepared extemporaneously because of the unavailability of licensed preparations for adults who cannot swallow tablets or for individuals that require a lesser dose [2].

14.2 Need of Extemporaneous Preparations

The extemporaneous pharmaceutical compounding has re-emerged the specialized pharmacy services mainly in the United States and Australia. However, the exact reason for the growth in this practice is still not known [4]. Extemporaneous preparations are usually prepared for patients that require special care in hospitals and community. It includes neonates, children, older patients, patients who undergone stroke, or patients relying on nasogastric intubation. The extemporaneous preparations for pediatrics are mainly formulated because of lack of commercial products, and mostly drugs are not labeled for children use. This might be due to a limited target market and strict legal liabilities. In such cases, liquid dosage forms are prepared from the solid dosage forms to meet the needs of individual patients [5]. However, in general, the extemporaneous preparations are prepared due to (a) lack of required strength, dose, and dosage form; (b) dilute medicines for

pediatrics and geriatrics; (c) convert solid oral dosage forms into liquid dosage forms; and (c) mix topical products that are commercially unavailable and (d) to avoid the excipients that may cause allergy in individual patients [6].

However, the extemporaneous preparations possess major risks to patient safety which includes quality (product quality and stability), environment (clean and isolated compounding area to ensure particulate-free airflow), personnel activities (human error, expiry date check, adherence to protocols), and the control process (maintaining regular surveillance over the preparation process [7]. Moreover, several errors related to extemporaneous preparations are reported merely because the pediatric patients are incapable of informing any adverse reaction, for example, “the peppermint case”; the use of incorrect strength of chloroform water caused the death of a child in the United Kingdom. Similarly, the overdose of imipramine and clonidine in extemporaneous preparations resulted in the deaths of children in the United States [2]. Therefore, the extemporaneous formulation prepared must contain a limited proportion of excipients especially for pediatrics. Dilutions of simple syrup are most commonly used as a vehicle for extemporaneous preparations for pediatric use [8].

14.3 Compounding Standards for Extemporaneous Preparations

The extemporaneous preparations are considered safe, accurately prepared, and appropriate for use by the patients. Therefore, while formulating an extemporaneous preparation, the following rules must be followed:

- An extemporaneous preparation is only prepared if there is no such commercially available product and suitable facilities meeting the standards are available for preparation.
- The pharmacist and staff should be trained and experienced to do such a task.
- The facilities and equipment required for compounding are readily available and kept in good condition.
- The pharmacist should be well aware of the required quantity and safety of the product.
- The raw materials should be from a recognized manufacturer and of required standards. Moreover, the set regulations must be followed.
- Special attention should be given for hazardous substances and must be handled appropriately.
- The extemporaneously prepared product must be labeled properly. The expiry date and storage condition must be mentioned on the label.
- The record of product must be kept for at least 2 years for traceability.

14.4 Commonly Compounded Extemporaneous Products

The extemporaneous products are divided into two classes depending upon the type of compounding:

- **Simple**: It includes topical creams, ointments, lotions, gels, oral liquids, capsules, tablets, powders, suppositories, and pessaries. These preparations do not require specialized facilities to be prepared.
- **Complex**: It includes parenteral ingredients with a safety hazard, single-unit microdose, modified-release dosage forms, and ophthalmic preparations. Proper training, adequate facilities, and specialized equipment, i.e., laminar flow hood or dry heat ovens, are required for compounding these products [1].

14.5 Current Scenario of Extemporaneous Pharmaceutical Products

The extemporaneous preparation is considered as a most risk preparative activity performed in the pharmacy as it involves compounding risk as well as unlicensed medicine risk**.** However, due to lack of research and development in this field, a potential risk of poor dose uniformity or formulation failure occurs, which may render product inefficacious and toxic [2].

14.6 Stability Studies: An Introduction

The stability studies of pharmaceutical products are a set of multiple processes that require substantial time, cost, and competence to provide a safe and effective medication. The most critical step during product development is to ensure the purity and stability of the pharmaceutical product. Stability is defined as the extent to which the remains within its defined physical, chemical, microbiological, and toxicological limit throughout its shelf life. Thus stability testing assesses the effect of physical and chemical factors on the quality of pharmaceutical products. Furthermore, the information obtained from stability studies plays an important role in the regulatory approval of the product [9].

14.7 Importance of Stability Studies

Stability testing is performed primarily to ensure the effectiveness and efficacy of the product. Besides the product degradation and formation of toxic metabolites, the unstable product may lead to loss of activity up to 85%. This may lead to therapy failure resulting in even death, e.g., loss of activity of nitroglycerine tablets used in angina may cause serious harm to the patient. As a consequence of this, it is necessary to perform stability studies and provide data prior to the approval of the

pharmaceutical product. Moreover, the stability studies will safeguard the prestige of the manufacturer by ensuring that the product will remain stable throughout its market period and shelf life. Furthermore, the stability studies provide database about the selection of appropriate formulation, excipients, and primary packaging material for the development of a new product, to assess the shelf life of new product and preparation of dossier, to justify the declared shelf life, and to substantiate that no changes occurred in the product that can affect its stability [10].

14.8 General Protocol for Conducting Stability Studies

A written document is established prior to initiating a stability testing of the pharmaceutical product. The protocol for stability study is designed depending on the type of the drug substance, dosage form, and container. Moreover, it also depends if the product is new in the market or not [11]. The protocol also highlights the regions in which it will be marketed. For example, if the product is proposed to be marketed in multiple climatic zones like I–III and IVb, then all these zones must be mentioned in the protocol. A practical well-designed stability protocol should contain information about the product batches to be included in the study, appropriate containers and closures, orientation of storage of containers, sampling time points, and a detailed sampling plan [9].

14.9 Stability of Extemporaneous Pharmaceutical Products

Whenever an extemporaneous preparation is required, the pharmacist must choose a suitable formula. The methods/formula utilized for extemporaneous dispensing should be preferably validated and have sufficient stability evidence. These pieces of evidence should be kept in record, and references must be noted on individual worksheets [2]. Each extemporaneously prepared dosage form possesses distinct stability testing. The stability studies of extemporaneous preparations are discussed below briefly with suitable examples:

14.9.1 Solutions

Despite the ease of administration and rapid absorption of the drugs in solution form, drugs tend to degrade rapidly in comparison to solid dosage forms and are more liable to microbial contamination as compared to suspensions. Moreover, the bottles used for packaging are of considerable size and inconvenient to carry. These factors may give rise to noncompliance with the pharmaceutical product [2].

Example 1 Ziprasidone, an antipsychotic drug, is effective in the treatment of tic disorder in children (7–16 years) when given in doses of 5 to 20 mg everyday [12]**.** Commercially, a 20-mg capsule of ziprasidone is available due to which

administration of smaller doses is a serious concern. However, a liquid preparation of dose less than 20 mg was prepared with stable chemical and physical properties as well as ease of administration. Geodon for injection (ziprasidone mesylate, 20 mg/ml) was diluted to 2.5 mg/ml using commercially available sugar and alcohol syrup. This preparation underwent stability testing by storing at room temperature under light, at room temperature in darkness, and in the refrigerator. The samples were drawn at different time intervals, and stability was checked utilizing high-performance liquid chromatography (HPLC). The ziprasidone syrup that was stored in the refrigerator retained its 90% potency for almost 6 weeks. However, syrups stored under other conditions were comparatively less stable. Therefore, the long-term compounding of ziprasidone syrup is suggested instead of daily compounding as it retains physical and chemical properties under refrigeration for 2 weeks. The syrup is recommended to be stored under refrigeration at 5°C [13].

Example 2 Solutions of acetylcysteine were prepared in two concentrations, i.e., 1% and 10%, for the treatment of meconium ileus. These solutions were prepared using commercially available 20% acetylcysteine solution containing bacteriostatic and 0.9% sodium chloride for injection. Three samples were prepared of both concentrations and stored in amber plastic bottles at 20–25°C. Samples were taken at 7, 14, 30, 60, and 90 days, respectively, and stability was checked using high-performance liquid chromatography. Initially, no change was observed in both concentrations. However, after 90 days, the pH of both concentrations changed and a pungent smell was observed. It means neither solution was stable up to 90 days. Therefore, it is concluded that the extemporaneously prepared acetylcysteine solution with bacteriostatic at concentrations (1% or 10%) remains stable for 60 days, if stored in an amber plastic bottle at room temperature [14].

14.9.2 Suspension

While preparing a suspension, special attention is required to achieve uniform particle size and dispersion. This can be achieved either by using raw material of acceptable standards or by grounding the tablets into a fine powder. This is considered to be the critical step in preparation of suspension, and quality checks are performed to assure uniform dispersion. Commercially available suspending agents such as xanthan gum can be used for the insoluble excipients of tablets and capsules. However, care should be taken that the suspending agent is also compatible with the drug. Considerable data is available to provide information regarding the compatibility of drugs with suspending agents [2].

In case of suspensions, physical stability is equally or even more important than chemical stability. This is because suspensions exist in more than one state (liquid and solid), which results in multiple physical and chemical degradation pathways. However, this fact is generally less attended while preparing an extemporaneous suspension [15].

Example 1 Rosuvastatin is commercially available only in solid dosage forms. However, if a liquid dosage form is prepared, it may ease the administration for those patients who are unable to swallow tablets. An extemporaneous suspension of rosuvastatin 20 mg/ml was prepared and stored under various conditions for 30 days. It was observed that the suspension stored at room temperature was more stable in comparison to other formulations [16].

Example 2 Enalapril maleate is used in the treatment of essential hypertension and congestive heart failure in children. It is commercially available only as 5 mg, 10 mg, and 20 mg tablets. There is unavailability of enalapril maleate suspension in the market for children. Mostly enalapril tablet is grounded and packed in capsules for children. The capsules are then emptied into liquid food for administration. However, the dose accuracy and taste masking are not achieved, resulting in poor patient compliance. Therefore, the extemporaneously prepared enalapril oral suspensions of 0.1 mg/ml and 1 mg/ml concentrations were prepared and evaluated. The suspensions were prepared using both sugar-free and sugar-containing vehicles. It was observed during stability testing that both the suspensions remained stable for 30 days at 40°C and 25°C and no change in viscosity was seen. Almost 98% of enalapril maleate concentration was maintained throughout this period. Hence, this provides a more suitable dosage form for pediatric patients [17].

14.9.3 Ointments

A considerable portion of extemporaneous prescriptions consists of topical preparations specifically in the field of dermatology. Different ointment bases possess distinct properties, and drugs are added to them in the form of aqueous or alcoholic solutions. However, there is no guideline that indicates the water-absorbing capacity of ointment bases. Dermatological ointments are often prepared extemporaneously and require specific storage to retain stability.

Example 1 Hydroquinone, a skin depigmentation agent is extemporaneously prepared in hospitals. It was reported that chromatic aberration emerges in ointments after a time that leads to noncompliance. Therefore, the effect of different storage conditions was evaluated on hydroquinone ointment using a spectrophotometer. It was observed that aberration was greater at high temperature and light while it was reduced when stored at 4°C. Consequently, it was concluded that it is highly important to guide patients regarding its storage conditions. It is instructed to store hydroquinone ointments at low temperature and always close the cap tightly after every use [18].

Example 2 Methyl salicylate ointment is prepared extemporaneously in hospitals as an analgesic ointment. The stability study for it was performed at various temperatures, i.e., 25°C±5°C and 2°C–8°C for 120 days. It was observed that the methyl salicylate ointment remained stable for 131 days when stored at room

temperature. However, the ointment remained stable for 176 days when stored in the refrigerator. Therefore it is recommended to store the extemporaneous methyl salicylate ointment at low temperatures up to 4 months [19].

14.9.4 Tablets

It is often observed that tablets are dispersed in solutions to get the desired dosage form, but it leads to an inaccurate dose. Similarly, grounding tablets with specific coating may also lead to inefficacy of active ingredients. Therefore, care should be taken while formulating tablets extemporaneously.

Example 1 A fast-dissolving tablet is a novel choice for extemporaneous preparation of dehydroepiandrosterone. Despite the ease of administration, there are limitations with the physical and chemical properties over long-term storage time. Therefore, the 10 mg dehydroepiandrosterone rapid-dissolving tablets underwent stability testing for over 6 months. The tablets were initially heated at 100°C for 30 minutes and then cooled at room temperature prior to packing in amber-colored blisters. The tablets were stored at 25°C and 60% relative humidity. No changes were detected by visual inspection, by weight check, or even by high-performance liquid chromatography in the physical and chemical properties of the tablets. Therefore, the rapid-dissolving tablets of dehydroepiandrosterone are stable when stored at 25°C and 60% relative humidity for up to 6 months [20].

14.10 Stability Criteria and Beyond-Use Dating

Beyond-use dating (BUD) is defined as the date after which an extemporaneous preparation should not be used. It is calculated from the date on which the preparation was compounded. As the extemporaneous preparations are considered to be used immediately, the criteria to determine BUD differs from the expiry date of commercially manufactured products. When determining the BUD, the following points should be considered:

- The drug nature and its degradation mechanism
- The dosage form and components
- The chances of microbial contamination in the formulation
- The primary and secondary packaging material in which drug is packed
- The expected storage condition
- The intended duration of use

However, if the commercially available product is used as an active ingredient for compounding, BUD is not determined only on the basis of the expiry date of the product. Instead, the pharmacist will take all information regarding stability, compatibility, and degradation under consideration [21] .

14.11 Conclusion

In conclusion, the stability of extemporaneously prepared products is equally important for commercially manufactured products. Good manufacturing practices are followed during the large-scale production of pharmaceuticals in pharmaceutical industries; hence good laboratory practices should also be followed during extemporaneous compounding of a product in pharmacies or hospitals. Such pharmacies should put protocols of the United States Pharmacopeia for pharmaceutical compounding of non-sterile and sterile preparations into practice. It is the responsibility of compounding pharmacists to adhere to these protocols and ensure the stability and efficacy of the compounded products.

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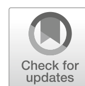

Stability Studies of Parenteral Products 15

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Abstract

The stability studies of parenteral products are one of the very significant criteria for the new drugs' formulation. Stability studies confirm that the quality, safety, and effectiveness of drug products are preserved all through the shelf life as an essential requirement. The stability tests must be performed subsequently according to the regulations given by the International Council for Harmonization, the World Health Organization, and/or other agencies. This chapter discusses the importance of stability studies, stability testing techniques, and specifications on stability studies with other factors. It provides a summary of the various stability study types that validate the stages of drug development. Furthermore, the chapter will discuss the framework of the ICH guidelines and also the importance of analytical techniques in the stability of parenteral products.

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_15

Keywords

Parenteral · Stability · Stability testing · ICH guidelines · Validation

15.1 What Are Parenteral Products?

Parenteral products are generally described as sterilized solutions, suspensions, emulsions, and powders for injections; these formulations are given directly into the tissue of the human body, skin, and mucous membranes by primary protection systems [1]. The word "parenteral," which is used for the preparation given through injection or infusion into one or additional skin tissue layers, is taken from the Greek term "para" and "enteron," which mean outside the gut and used for the dosage forms given by routes besides the oral route. Some drugs can only be given parenterally, specifically peptides, proteins, and several chemotherapy drugs because when given orally, they are denatured in the gastrointestinal tract. Parenteral preparation must be purified and exempt from any physical, chemical, and biological contaminations. They must be compliant with intravenous diluents, administration process, as well as additional co-administered drugs [2]. The parenteral preparations are administrated into the body by injecting, e. g., directly, bolus intravenous, intramuscular, or subcutaneous, or by infusion with a controlled rate or by direct implantation through intramuscular or subcutaneous. The different routes of administration for parenteral formulations are:

Intradermal: These are incorporated into the skin between both the dermal and the epidermal layer.
Subcutaneous: The drug is injected into the tissues between the skin and the muscles.
Intramuscular: The drug is injected deep into the muscles.
Intravenous: Drug is injected directly through the veins into the bloodstream.
Intra-arterial: Drug is injected into the artery for an immediate peripheral effect.
Intra-cardiac: The injection places the drug directly into the left ventricle.
Intrathecal: Drug is administered into the subarachnoid space of the spinal cord.
Peri-dural: Drug is injected between the dura matter and the inner aspects of the spine.
Intra-articular: Drug is injected into the synovial fluid in a joint [3, 4].

15.2 Why We Formulate These Products and What Are the Limitations?

Parenteral products are developed to avoid the most guarding barriers of the body skin and the mucosa; hence, these must be "essentially" contamination-free. Most of these are injected into body tissues and enter the bloodstream without passing through the liver. This may include injections and topical and inhalation routes [5]. Several drug products can only be administered directly via injection because

when taken orally, they become denatured in the gastrointestinal tract. Parenteral drugs are generally unstable and extremely strong, requiring strict monitoring of patient administration [6]. Parenteral formulation is used to cause localized effect, administer drugs to an insentient patient, and assure that the drug is delivered to the targeted tissues.

Parenteral preparations are distinct from other dosage forms because they are directly entered into the bloodstream. They must be aseptic and free from all kinds of contamination. Such standards put a greater responsibility on the pharmaceutical industry to follow current good manufacturing practice (cGMP) in parenteral product preparation and to pharmacists as well as other healthcare professionals to perform good aseptic practice in the administration of parenteral products to patients [7]. Although parenteral drug delivery can often be confusing and associated with concerns including a small number of suitable excipients, strict standards for aseptic manufacturing, safety concerns, and patient non-compliance, this route retains its importance due to various benefits such as the rapid onset of action in emergencies [5, 8].

15.3 Types of Parenteral Products

Parenteral products can be listed as small-volume, large-volume, and lyophilized products [9]. These are sterilized products which are delivered into the body through multiple routes, through injections, infusions, and implantations. Several types of parenteral products can be distinguished [28].

15.3.1 Injections

Injections are aseptic solution, emulsion, or suspension. Injections are formulated by disintegrating and emulsifying the active material and additional excipients either in water for injections, in a compatible clean nonaqueous solvent, or a combination of such components. The solution to be injected can contain sediments that can be readily distributed by shaking the container. Suspension should stay stable to give a homogeneous dosage when extracted from the bottle.

15.3.2 Infusions

These are aseptic aqueous solution or emulsion; typically, they are isotonic with blood and mainly designed for large-volume dispensing. Infusion does not carry added antimicrobic preservatives.

15.3.3 Concentrates for Injections or Infusions

These are aseptic solutions designed for injection and infusion upon dilution. The concentrates are diluted to a prescribed volume with the prescribed liquid before dispensing. They meet the criteria for injection and infusion when diluted.

15.3.4 Powders for Injections or Infusions

Powders for injections or infusions are solid, aseptic substances that are dispersed in their final bottles and which, upon shaking with the prescribed volume of the sterile solvent, quickly form either a transparent and particle-free solution or a homogenous suspension.

15.3.5 Implants

They are sterilized solid formulations of a size and form ideal for parenteral insertion, which release the drug for a more extended period. Each dosage is given in an aseptic bottle [10, 11].

15.4 Stability Issues

Stability is determined as the period in a certain storage environment in a particular container-closure system where the substance maintains all its original characteristics within predetermined limits. The stability is defined in US Pharmacopeia (USP, 2016) [12] as the degree to which the drug stays in defined limits and through the storage and time of utilization, with similar characteristics and qualities which it had at the time of its production [13]. The stability of parenteral products must be maintained so that the desired dosage is given to patients. Hydrolytic and oxidative drug decay is the most common form of instability, but it rarely shows as cloudiness, precipitates, or color changes. The hydrolysis rate may be altered by the storage temperature or solution's pH. Oxidation is affected by temperature, pH, light, oxygen concentration in solution, impurity, and concentration of the oxidizing substance. Other degradation types can also happen in the solution. The selected stability method should have the ability to view the breakdown products as well as the original entity. Attempts should be made to force degradation and assess the changes to the original entity. To determine stability, it must be known what instability looks like [14]. Since drug stability is affected by several factors, pharmacists should use a short beyond-use date (BUD) to compound parenteral from bulk chemicals or know from the literature that more extended stability exists. The packaging selection is also important for parenteral drug stability [15].

15.5 Stability Evaluation

Stability studies aim to set up at least three batches of drug products based on testing and analyze the stability data (comprising, where applicable, physical, chemical, biological, and microbiological study results) and a retesting time relevant to the entire later drug product batches developed within the same conditions. The variability extent of individual batches influences the assurance that the later production batch should follow the guidelines through the allocated retest time (ICH Guideline, 2003) [16, 17]. The layout of the drug product stability study should focus on the analysis of all determinants, which can lead to physical, chemical, or biological changes over the suggested storage time. Tests for the drugs and the degeneration products and assessment of pH alteration, color, and appearance should be included. The drug stability evaluation facilitates the production of safer and more effective formulation, suitable packaging selection, setting up proper storing conditions, and the allocation of shelf life [17].

15.6 Stability Testing

Stability testing is a significant element of the drug development procedure and a prerequisite for the approval of drug products. The International Council for Harmonization (ICH 2003), the World Health Organization (WHO 2009), and the Food and Drug Administration (FDA 1998, 2014) have issued the stability testing guidelines for new drug products, including long-term, medium-term, and rapid stability tests. Stability tests aim to show how the quality of drug products changes over time due to several environmental factors, e. g., temperature, moisture, and light, and to set a reset timeframe for drug products or shelf life of drugs also prescribed during storing condition [16, 17].

15.7 Chemical Kinetics Involved in the Stability Issues

Chemical kinetics analyzes the rates and factors that affect chemical reactions. The stability studies of drug products involve the analysis of drug chemical deterioration reaction kinetics in a dosage form. The shelf life of a product should be determined in specified storing conditions. Drug product has different chemical structures and under different conditions with different orders of reaction follows one or more mechanisms of degradation. The most common drug degradation reactions involve oxidation, hydrolysis, and also photolysis. Such reactions can take place when the drug product is manufactured, stored, and used. To ensure the safety of the patient, the pharmacist must be conscious of the impacts of such reactions on efficacy loss and of product toxicity development. The stability-indicating assay method (SIAM) can evaluate the degradation compounds that can also give a precise efficacy loss assessment of drug products. To assess the shelf life of a drug product and determine the expiry date, SIAM data are then put through kinetic treatment. A reassessment of

the packaging and storage condition may be required if the formulation specifications are altered to increase product stability. A clear understanding of the basic principles of chemical kinetics is required to determine the degradation reaction rate of the drug product and predict the shelf life and expiry date. The kinetic parameter can help elucidate the degradation reaction mechanism and, therefore, allow proper measures to stabilize the product [17].

15.7.1 Factors Affecting the Stability of the Parenteral Products

The drug products' physical and chemical properties are affected by multiple factors. These factors affect the stability of various dosage forms while manufacturing and storing and are defined as the external and internal factors.

15.7.2 External Factors

External factors contain temperature, sun, humidity, oxygen, carbon dioxide, as well as microbiological contamination. The use of suitable packing material and storage conditions can accommodate external factors.

15.7.3 Internal Factors

Internal factors involve pH, solvent, buffer type, particle size, and drug-drug, drug-excipient, and drug-bottle interaction. By choosing optimal formulation parameters to maintain an appropriate degree of stability, the effect of internal factors can be reduced [17].

15.7.4 Standards and Guidelines for Stability Study of Parenteral Products

In 1992 ICH Stability Testing Guidelines (Q1A) were issued, which were accepted across the ICH area, that is, the European Union, the USA, and also Japan. Other regions, such as Australia, Canada, and Switzerland, accepted the guidelines in principle. This guideline aims to demonstrate the key stability data plan for the new drug product; however, it allows ample flexibility to cover a range of empirical situations which can occur as a result of certain scientific consideration and material characteristics to be tested.

15.7.5 ICH Q1A: Stability Testing of New Drug Substances and Products

The selection of testing conditions set out in the guideline is established on a study of atmoshpheric condition effect in the three regions of EC, Japan, and the USA. The climate data can be used for calculating the average kinetic temperature in any region of the globe and dividing it into four I–IV climate zones. This guideline discusses the climate zones I and II. The concept has been developed to ensure that the stability data given to any of the three EC regions, Japan, and the USA should also apply to other two areas, mutually, given that the data is following the guideline and that the labeling conforms to national and regional criteria [16].

15.7.6 Q1B: Photostability Testing

Before ICH guidelines, protocols and instruments were not systemized or generally utilized to measure the photosensitivity of a drug product. Therefore, experts from Japan were necessary to address optimum photo sources that stimulate sunlight as well as techniques to calculate the light intensity. As a response, in November 1996, the tripartite harmonized ICH guideline (Q1B), photostability testing of new drug substances and products, was annexed to a parent stability guideline [18]. This ICH guideline has helped in strategy standardization. An overview of ICH guidelines describing specific terms in photochemistry, examining photostability analysis and photo source characterization, and also calculating the results from the source of photolysis used for photostability testing in pharmaceutical industries is given by Thatcher et al. [19]. Table 15.1 outlines the ICH guideline codes and descriptions.

Table 15.1 Codes and titles covered under ICH guidelines. (Adopted from Ref "Saranjit. S, Monika. B, 2000")

ICH codes	Guidelines
Q1A	Stability testing of new drug substances and products (second revision)
Q1B	Photostability testing of new drug substances and products
Q1C	Stability testing of new dosage form
Q1D	Bracketing and matrixing designs for the stability testing of drug substances and products
Q1E	Evaluation of stability data
Q1F	Stability data package for registration applications in climatic zones III and IV
Q5C	Stability testing of biotechnological/biological products
Q6A	Specifications: test procedures and acceptance criteria for new drug substances and new drug products – chemical substances
Q6B	Specifications: test procedures and acceptance criteria for new drug substances and new drug products – biotechnological/biological products

15.7.7 Q1C: Stability Testing for New Dosage Forms

The TH-ICH Q1C guideline was adopted during 1996. This broadens the fundamental stability guidelines for a new formulation of previously authorized drugs and specifies the conditions in which decreased stability data may be approved. This the most concise of all other ICH guidelines to date. It is because ICH authorities could not decide on the level of supporting data of identical drugs and products or the same dosage forms, which can enable producers to minimize the new formulation stability testing [16].

15.7.8 Q1D: Bracketing and Matrixing

Q1D guideline outlines the concept of decreased stability testing and gives bracketing and matrixing design description. The adoption of this strategy by authorities saves producers a large number of needless stability testing. Limited data, on the contrary, indicates a higher chance that the outcomes produced might not be large enough to maintain the anticipated shelf life [20].

15.7.9 Q1E: Evaluation of Stability Data

In February 2003, TH-ICH Q1E guideline was concluded. Q1E guide broadens the fundamental guideline by describing possible scenarios in which retest period/shelf life extrapolation over real-time data can be acceptable. It also gives an example of analytical techniques for stability data studies [21, 22].

15.7.10 Q5C: Stability Testing of Biotech Products

Since the majority of proteins and polypeptides are unstable compared with micromolecules and since assay protocols and deterioration products are distinctive, the ICH committee decided at an early stage to encourage biotech experts to establish specific guidelines for such product types. The tripartite harmonized ICH guidelines on (QC5) stability testing of biotechnological/biological products was published in November 1995 [23].

15.8 Guidelines

15.8.1 Drug Substance

Stability drug product data is an essential element of the formal stability analysis method.

15.8.2 Stress Testing

Stress testing is essential to analyze the drug substances and products in different circumstances of higher temperatures and moisture. Stress testing data can help to understand the stability profile in production, storage, distribution, and patient usage. Such testing gives insight into possible deterioration products and helps to identify the pathways for deterioration [16, 24].

15.8.3 Batch Selection

Stability test data should be given not less than three batches of drug products. The production of batches should be along the same synthetic course at a minimum pilot scale utilizing manufacturing procedure, and a technique simulating the terminal procedure can be used for manufacturing batches. The general standard of product batches used in the validated stability tests should be indicative of material quality manufactured on the production scale [16].

15.8.4 Container-Closure System

Stability tests should be performed on drug products packed into a container-closure system, which is similar to or replicates the suggested package for storage and supply.

15.8.5 Specifications

Specifications are explained in ICH Q6A and Q6B as a series of tests relating to analytical methods and the proposed terms of approval. In Q3A, the product degradation specifications for a drug substance are also addressed. Stability studies should also contain an analysis of those drug product characteristics subjected to alter amidst storage period and are anticipated to have an impact on quality and effectiveness. The tests should involve, as needed, physical, chemical, biological, and microbiological properties. The outcomes of the validation studies rely on whether and to what extent replication is to be done [16, 25].

15.8.6 Testing Frequency

The guideline suggests testing every 3 months of the first year, every 6 months of the second year, and yearly afterward. This means a total of three time points (together with starting and ending time points) are needed for accelerated and four time points for the moderate condition. Minimal three time points, with starting and ending points (0, 3, 6 months), from a 6 months' study, are suggested for accelerated

storage. Where an assumption (derived from developmental experience) which outcomes from rapid studies are potentially approach substantial change thresholds, additional testing should be done by increasing sample at the last point time or by point four in study design. A total of four time points (0, 6, 9, 12 months), with the starting and ending times points from a 12-month study, is suggested. At the same time, testing at moderate storage conditions is required due to substantial changes in accelerated storage.

15.8.7 Storage Conditions

In the ICH cycle, a number of suitable storage requirements are harmonized in zones I and II. To assess the stability of drug products, an amalgamation of temperature and moisture is essential. Besides, the guideline describes the scope of temperature and moisture parameters for storage chamber control: The temperature of the compartment should be managed within ±2 C and moisture should be within ±5% of the relative humidity. The drug products are stored at room temperature, and the guideline specifies a median temperature of 30 C/65% RH testing in this median state is required if there is a substantial change in samples that are stored for 6 months in rapid RH 40/75% condition. Significant changes are noticed for the drug product when any evaluation of rapid samples does not comply with room temperature requirements. This analysis must be done promptly in order to pull the stored samples from the chamber and to be examined immediately.

15.8.8 Stability Commitment

ICH guideline commends that stability commitment should be included in the registration application. It requires the candidate to carry out stability tests for three commercial batches following the current protocol via projected shelf life. The guideline also states that the stability method used for long-term stability commitment studies for primary batches should be constant. Thus, when there is a considerable variation in the accelerated condition of primary batches, samples of intermediate condition should be checked, and samples from three production batches of intermediate condition should be tested as well.

15.8.9 Data Evaluation

The ICH guideline suggests that the data assessment should be conducted for the submission of batches. It also explains that no systematic statistical analysis is important if data indicate any no deterioration or variation. A reason for exclusion is requisite to prove that the data set stays under the range of the method and does not indicate any specific trend over time.

15.8.10 Labeling

A storage document for labeling should be implemented following the applicable national or regional criteria. The emphasis should be on evaluating the stability of drug substances. Clear instruction should be given, where applicable, especially for drugs that cannot tolerate freezing. The time for a retest should be determined from stability data, and where applicable, the container label should have a retest date on it [16, 21].

15.8.11 Other ICH Guidelines

15.8.11.1 Drug Product

The layout of structured stability studies of a pharmaceutical product should build on the understanding of actions and characteristics of drug substances, stability studies, as well as expertise of clinical formulation study. The possible changes in storage and reasoning for the selection of properties to be evaluated in structured stability studies should be mentioned.

15.8.11.2 Photostability Testing

Photostability tests should be performed with minimum one primary drug product batch, as needed. The basic criteria for photostability tests are set out in ICH Q1B [26].

15.8.11.3 Selection of Batches

Formal stability test data should be given for a minimum of three primary batches of the drug product. The batches should be produced at a small pilot scale along the same synthetic route as the production batches by a manufacturing and processing system which replicates the final procedure, which is used for production batches. The general quality of drug product batches used in the formal stability testing should indicate the quality of the material manufactured next at commercial scale. Additional data support may be given.

15.8.11.4 Container-Closure System

Stability tests should be performed on a drug product packed into a container closure system, which is similar to or replicates the suggested package for storage and supply.

15.8.11.5 Specification

Specifications are explained in ICH Q6A and Q6B as a series of tests relating to analytical methods and the proposed terms of approval: test procedures and acceptance criteria for new drug substances and new drug products, chemical substances (Q6A), and drug products, biotechnology/biological products (Q6B). In Q3A, the product degradation specifications for a drug substance are also addressed. Stability studies should also contain an analysis of those drug product characteristics which

are subject to alter amidst the warehouse period and are anticipated to have an impact on quality and effectiveness. The tests should include, as needed, physical, chemical, biological, and microbiological characteristics. The outcomes of validation studies rely on whether and to what extent replication is to be done [25].

15.8.11.6 Testing Frequency

The test frequency should suffice to evaluate the stability profile of the drug product in long-term studies. For drugs with a recommended retest minimum period of 12 months, testing should usually be every 3 months of the first year, every 6 months of the second year, and yearly afterward. This means a total of three time points (together with starting and ending time points) is needed for accelerated and four time points for the moderate condition. Minimal three time points, with starting and ending points (0, 3, 6 months), from a 6 months' study, is recommended for accelerated storage. Where an assumption (derived from developmental experience) which outcomes from rapid studies are potentially approach substantial change thresholds, additional testing should be done by increasing sample at last point time or by point four in study design. A total of four time points, with the starting and ending time points (0, 6, 9, 12 months), from a 12-month study, is suggested. At the same time, testing at moderate storage conditions is required due to substantial changes in accelerated storage.

15.8.11.7 Storage Conditions

The drug product should generally be assessed within storage condition (with suitable tolerance) that evaluates its thermal stability and also moisture sensitivity, where necessary. Long-term testing should comprise at least 12 months for a minimum of three primary batches at the time of submission and should be extended for some time sufficient to cover the planned retest period. If required, supplemental data obtained through the evaluation of application registration can be given to the authorities.

15.8.11.8 Stability Commitment

If long-term stability data for primary batches do no incorporate the suggested retest timeframe upon approval, to develop a firm retest period commitment shall be made to pursue post-approval stability studies. A commitment after approval is deemed needless when the submission requires long-term stability data for three production batches comprising the suggested retesting duration. Therefore, one of these obligations should be met:

If data from stability studies for a minimum of three production batches are included in this submission, a commitment to perform these studies during the recommended retest period should be made. If the submission contains stability study data from less than three batches, a commitment to continue these studies during the recommended retest period should be made, and add supplementary production batches to a total of three batches to a total of at least three batches, on long-term stability studies during recommended retest period.

15.8.11.9 Evaluation

The approach for interpreting the quantitative attribute data expected to alter over a period is to measure the time at which a 95% unilateral dependence limit for the average curve crosses the acceptance criteria. If the data show a high batch-to-batch variation, the data should be merged into a cumulative estimate, and it is desirable to merge the data into a total estimate. It can be undertaken by implementing suitable statistical tests first, such as p values with a significance level higher than 0.25 at regression line slope and zero time intercept for each batch. If combining the data of several batches is not appropriate, the average retest cycle should be based upon the least time that the batch can anticipate to stay under the acceptance criteria. The type of any deterioration association would decide if the data should be converted to linear regression analysis. In general, the association can be expressed by a linear, quadratic, or cubic form on a + 6 arithmetic scale. Statistical methods should be used to check the data quality of all batches and mixed batches (if applicable) of the expected deterioration curve. Restricted real-time data observation from a long-term storage condition above the range identified to broaden the retest duration may be performed at the time of approval if appropriate. For example, the reasoning should build on what has been known regarding the deterioration process, test result within the accelerated condition, quality of the mathematical model, batch size, or supporting stability data. Nevertheless, such observation implies that the same deterioration relation would be applied further than identified data. The analysis should comprise not just the test but also the extent of the deterioration product and other relevant parameters.

15.8.11.10 Labeling

A storage document for labeling should be implemented following the applicable national or regional criteria. The statement should focus on evaluating the stability of drug substances. Specific instruction should be given, where applicable, especially for drugs which cannot tolerate freezing. The time for a retest should be determined from stability data, and where applicable, the container label should have a retest date on it [16, 21].

15.8.12 Analytical Techniques for Stability Studies of Parenteral Products

Analytical techniques, from drug development to marketing and post-marketing, play an essential role to determine the drug's physical and chemical stability, evaluating the stability of drug molecules, indicating the drug's stability in the formulation and thus the shelf life particularly, and signaling the presence of the substance in its pure form or the existence of any contaminants (either as a drug precursor or degradation product as a result of chemical/photochemical effects). This can be done by using the stability-indicating assay method like high-performance liquid chromatography (HPLC), for the evaluation of preserved substance but also its degradation product. To achieve the required specification of a specific system,

the process should be validated. The stability of drug products produced in many dosage forms and kept in distinct packing can also be assessed with variable strengths [17, 27]. When presenting and evaluating the stability data, a structured approach should be followed that should include physical, chemical, biological, and microbiological test characteristics if required. All product characteristics, for example, assay value or effectiveness, the decomposition product material, and physicochemical properties, probably influenced by storage, should be determined. To determine if these additives stay active and stable across their anticipated shelf life, test methods for demonstrating the effectiveness of additives, for instance, antimicrobial agents, should be used. Analytical methods should be verified, and both accuracy and precision should be noted (standard variations). The test methods selected should be indicative of stability. The tests should be checked for related compounds or degradation products to indicate their specificity and their sufficient sensitivity to the product being tested [17]. The following chemical and microbiological tests are typically performed for the final products to ensure that each of the following criteria for a parenteral product is properly met.

Chemical testing involves:

(i) Identification test for the pharmaceutically active ingredient
(ii) Potency assay
(iii) Calculation of deteriorating product or impurities relating to the process
(iv) pH
(v) Osmolality
(vi) Appearance (color testing)
(vii) Assay for the contents of essential excipients and their main degradation products (e.g., dextrose and 5-hydroxymethylfurfural and ethanol)
(viii) Suspension and emulsion particle size distribution
(ix) Water content for lyophilized dosage forms

Microbiological testing includes:

(i) Sterility test
(ii) Microbial endotoxin test
(iii) Particulate matter test
(iv) Bioburden analysis (bulk)
(v) Container/closure solidity [9]

15.8.13 Advantages of Analytical Techniques

Modern analytical techniques provide the following advantages:

(i) Proper identification of drugs in bulk form or as a manufactured product
(ii) Indicate the proportion of the specified material of drug in formulation within a specified limit

(iii) Determine the drug's stability in the formulation and thus the shelf life particularly, the presence of the drug in its preserved form, or the presence of any contamination (as drug precursor or decomposition product because of chemical/photochemical factors)
(iv) Usage in the study of dissolution rates, i.e., at what speed the drug is released from its formulation for absorption into bloodstream (bioavailability studies)
(v) Confirm that the specification and sterility of drug (bulk form) fulfill official standards and monographs
(vi) Confirm that the specification and sterility of excipients used in formulation fulfill official standard criteria
(vii) Indicate the concentration of identified contaminants in the pure drug substance [28].

15.8.14 Disadvantages of Analytical Techniques

(i) Instrumental techniques are expensive due to cost and maintenance, and trained staff is needed for handling.
(ii) Sensitivity and precision rely on the instrument type.
(iii) The outcomes must often be checked using other techniques.
(iv) The instrumental approach might not be accurate in a particular situation.

15.9 Conclusion

Parenteral products are sterile products that are designed for direct administration into the systemic circulation of humans or animals. This chapter gives an understanding of stability studies and issues involved in instability testing and its importance. The stability studies have made the critical methodological contribution to the new drugs as well as new formulation development program, and it became easy to predict the shelf life and the effect of environmental factors for the product degradation. Any variation in the defined stability profile can affect its quality, safety, and efficacy. Stability tests are performed to add suggested storage and shelf life conditions to the label, and it is safe and efficient during its shelf life. They should comply with the pharmaceutical quality standards depicted in different pharmacopeias and ICH guidelines, assure clinical tolerance, and be safe for the desired use.

Conflicts of Interest The authors declare no conflict of interest.

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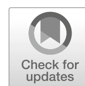

16 Stability Studies of Solid Dosage Forms

Ghulam Murtaza, Munazza Ijaz, Hafsa Anam, and Saba Shamim

Abstract

Pharmaceutical preparation mechanism is a continuous phenomenon. Hundreds of new solid formulations are developed every year. Thus, it is necessary to test and evaluate each solid dosage form for the accurate dispensing and safety of consumers. For this purpose, stability studies and chemical kinetic evaluation pattern must be observed to get the license for the delivery of new drug formulation. For the stability evaluation of solid dosage forms, ICH and WHO guidelines have to be followed, in which a series of tests is involved to prove the validity and rationality of solid dosage forms. Furthermore, the factors or issues related to stability are observed, and chemical kinetics of the solid dosage forms are monitored. The chemical kinetics involved in drug products from their preparation to storage cause chemical instability of drug formulation. This instability may be due to hydrolysis, photolysis, racemization, or other chemical reactions induced during preparation or storage. Thus, for this purpose, stability studies are applied to achieve the stable finished product. Degradation reactions constitute the reason of chemical instability. Thus, it is considered as the most important aspect during stability studies. Degradation processes affect the pre-formulation studies. If this process is not monitored, drugs stored in optimum conditions could be vigorously degraded. Thus, the stability testing is required to prevent any kind of unwanted effects and for the achievement of stable drug throughout its expected shelf life.

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M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_16

Keywords

Pharmaceutical preparations · Stability · Chemical kinetics · ICH and WHO guidelines · Chemical instability · Pre-formulation studies

16.1 Introduction

Drug stability is considered as an important factor for preparing pharmaceutical products. It is the property of pharmaceutical dosage form, which is essential for its pharmacological, microbial, physical, and chemical integrity during its storage and use. There are various properties of drugs, which are studied to investigate its stability: these are ionic strength, pH, and reaction rate constant under different environmental conditions [1]. Chemical kinetics is the physiochemical science to study reactions involved in different mechanism of actions and their engineering at the molecular level [2].

16.2 Types of Solid Dosage Form

Solid dosage forms are the most commonly used forms of drug that can be administered orally. The following are the types of solid dosage form.

16.2.1 Powders and Granules

Powders can be described as the pharmaceutical preparation comprised of dry mixture of finely divided drug intended for internal or external application. The solid materials present in powders are first characterized for their physiochemical properties such as particle size, solubility, and compatibility. Granules are agglomerates of powdered mixture [3].

16.2.2 Capsules

Capsule is an edible pharmaceutical preparation covered in a gelatin casing to be filled with medicine or inert substance [4].

16.2.3 Tablets

Tablets are solid dosage form manufactured by three different methods, viz., wet granulation, dry granulation, and direct compression method with or without excipients [5].

16.2.4 Solid Oral-Modified Release Dosage Forms and Drug Delivery System

Solid oral-modified release refers to the both delayed and extended release dosage forms intended for oral use and modifies delivery of poorly water-soluble drugs. The release of drug can be easily delayed or extended [6].

16.3 Stability, Their Types, and Factors Affecting Stability of Solid Dosage Forms

16.3.1 Stability

Drug stability is known as its ability to stay within its established specifications.

The drug stability influences the quality and safety of drug, which are tested for the rationality of the product. The stability tests applied on the product should be clearly stated [7]. Stability study and its testing are the essential part of the study of biological, physical, and chemical stabilities. The following are the types of stability studies:

16.3.2 Physical Stability

Many factors are involved in producing physical instability such as disintegration time, dissolution profile, loss of water and appearance, and absorption of water. To counter this instability, many evaluating processes are used for the rationality of the drug product. These include organoleptic evaluation of color or appearance of the formulation, uniformity, dissolution, and suspendability of the formulation. Physical stability affects shelf life and storage conditions of solid dosage form. It is a well-known fact that physical instability of any formulation due to any cause such as shaking, interaction with device, and temperature change can produce harmful effects on individuals after usage. The conventional testing process of visual investigation is also important but not enough. Therefore, additional sub-visual evaluation technique is also added for the rationality of the formulation.

16.3.3 Visual Evaluation

Visual investigation of any product allows to detect the following changes in the formulations. These are:

(i) Formation of particles >0.2 μm (optical examination: European Pharmacopoeia 7th edition test 2.2.1)

(ii) Change in initial color of the formulation (comparison of colors ranges from yellow to brown with the standard dilutions of red, yellow, and brown: European Pharmacopoeia 7th edition test 2.2.2 and 2.9.20)

Visual evaluation should be standardized according to the pharmacopeia monographs. In addition, turbidimetry and microscopic evaluation are reliable processes for physical testing of any formulation.

16.3.4 Sub-visual Evaluation

Some micro-precipitates, which are already present in formulation but not detected via optical examination and become detectable after some time especially at low temperature [7], are tested under sub-visual evaluation. The Lumry-Eyring model of nucleation theory clears that the drug molecules adsorb on any surface contaminated with microparticles during dilution process that results in the formation of microaggregates [8–12]. The formation of these aggregates is the sign of instability during the storage process. The following methods are adopted to maintain physical stability:

(i) Light obstruction (European Pharmacopoeia 7th edition, test 2.9.19)
(ii) Microscopic analysis (European Pharmacopoeia 7th edition 2.9.29)
(iii) Turbidimetry studies (three wavelengths at 350, 420, and 530 nm used to evaluate the microparticles)

Turbidimetry method is a non-destructive method when used at the above three wavelengths. This method can provide the estimation of the number of particles, if present in any formulation. This method has been used in protein aggregation process [13, 14].

Furthermore, stressed condition could also be applied to evaluate the physical instability such as stirring or shaking, which reveals the instability of thermodynamically unstable products [15].

16.3.5 Chemical Evaluation

Chemical stability can be defined as absence of any kind of chemical decay in the chemical entity found in the formulation in the form of excipient or preservative.

16.3.6 Degradation Methods

There are several methods involved, which cause chemical instability in the formulation; these are hydrolysis, oxidation, and photolysis. Thus, the following testing

methods are employed to assess any kind of chemical degradation in the formulation.

16.3.7 General Tests

The primary method to identify chemical instability is pH variation. This variation should be examined and measured till the end of the study. The data is recorded in a very careful manner. During this process, the main consideration should be to avoid any kind of degradation in the drug. The main cause of pH variation could be the CO_2 which can easily diffuse via the plastic wall container, or the other one can be the acidification of non-buffered solution, but this condition applies for acid-sensitive drugs, so no acidification is found if the drug is not acid sensitive. Thus, the pH variation should be explained even in slight modification of one or two units as it is a logarithmic scale, and one unit on pH means ten times increase in proton period pH concentration.

Chemical instability could be seen vigorously in the drugs warehoused in plastic bags, so diffusion of water vapors becomes the reason of water loss, so it is mandatory to achieve the correct amount of drug. Tested bags must be weighed at the time of sampling. If the loss is greater according to set standards, then major defects are needed to be considered deliberately, e.g., leakage problem or permeability problem. Thus, for better stability, the bag should be overwrapped to prevent water loss and from light absorption problem.

16.3.8 Methods for Evaluation of Chemical Instability

Stability-indicating assay methods (SIAMs) are potentially used quantitative analytical methods that can validate drug products to make them liable for practical use [7, 16]. These assays can find out the variation in drug properties such as physical, chemical, and microbiological, so that the contents found in active ingredients can be measured accurately. Thus, make sure that any vehicle or by-products achieved as a result of degradation do not cause any interference during the assay. However, the forced degradation studies are done on parent drug and are usually adopted to achieve chromatographic peak of degradation by-product or nature of the drug [17]. The most appropriate analytical methods are selected from already published literatures to prevent chemical structure degradation or its possible degradation pathway, though it must be considered that most of the analytical techniques are non-SIAM according to current guidelines [18]. Thus, to evaluate systematically, pharmacopeia started the analytical process from searching of primarily developed impurities during synthesis of raw chemical. The search of applicable methods of separation is necessary; thus, HPLC can be considered as a best method of separation. Other suitable methods of separation are high-performance thin-layer chromatography and capillary. Thus, to determine the drug products and the purity of the compound, peak spectral purity was applied to find out the online photodiode array.

Furthermore, mass spectrometry is also another technique used to determine drug products and its purity. To verify the above process, the validation of purity of peaks is done under stressed condition. Furthermore, along with above steps, stressed studies are strongly recommended for the stability of formulation. Spectrophotometric method along with titrimetric methods is usually recommended in particular cases.

16.3.9 Microbiological Stability

Microbiological stability study is used to assess that formulation is free of microbial contamination and is meeting the drug standards. The important factors to be elucidated during preparation and dispensing of the pharmaceutical formulation to avoid microbiological instability are:

(i) The preparation or storage of raw material in microbes' free environment
(ii) Drug manufacturing in microbes' free area
(iii) The fate of the contaminating microorganisms during storage

The main factor of contaminating raw material or pharmaceutical formulation is the bioburden of the formulations [19]. If these contaminated raw materials are used without any processing, it can cause severe infection, and on facing back, it was found that these were the raw material of natural origin found to be contaminated with microbial attack [20]. During tablets' manufacturing, dying process of granules [21] and compaction method [22] were found as the most susceptible method of contamination. However, solid dosage form such as tablets used starch and lactose which rarely contaminate with microbial growth. Water also plays important role in producing microbial instability; if the storage condition is not fully accomplished and there is some humidity in the environment, it definitely causes microbial growth in the formulation [23]. Thus in tropical regions where weather is hot and humid, the microbial growth increases vigorously with time due to climate condition; therefore the storage conditions should be ideal to restrain microbial growth. Additionally, several preservatives are used to control water absorption or loss to maintain product integrity [24].

16.3.10 Factors Affecting Stability Studies

The following are the factors affecting the stability studies of solid dosage forms [25]:

16.3.10.1 Temperature

Temperature is an important factor that influence stability of the drug substance as high temperature may cause oxidation, reduction, or hydrolysis which may lead to degradation of the drug product.

16.3.10.2 pH

Another contributing factor causing drug instability is the pH of the drug, as acidic or alkaline pH may determine the fate of the drug substance, which thus may cause degradation. Most of the drugs remain stable at pH 4 to 8. Drugs that are weakly acidic or weakly basic show good solubility in ionized form and readily decompose.

16.3.10.3 Moisture

Moisture in drug substance can play harmful role and is an important factor that causes drug instability. Moisture may cause microbial growth and catalyze reaction like oxidation, hydrolysis, and reduction reactions.

16.3.10.4 Light

Photosensitivity is an important issue for the drugs that are photosensitive; it employed its effect by energy or thermal effect.

16.3.10.5 Oxygen

Interaction of drug substance with oxygen can cause a series of oxidation reaction which can cause degradation of drug substance.

16.4 Limitations of Stability Studies

There are various limitations of stability studies [26]:

(i) The physical and chemical methods used to evaluate drug formulations are non-adapted even if they followed the ICH guidelines.
(ii) Those stability studies, which are based on Arrhenius equation, are only valid in the case if the breakdown is dependent on temperature.
(iii) The activation energy achieved in the study should be between 10 and 30 kcal/mole.
(iv) The emerging instability generated by stressed conditions.
(v) There is still a need for more rational and appropriate study design, such as sequential cycling or isothermal study, to validate the stability study.
(vi) The specific requirements involved in physical stability are not enough to authorize the physical stability of solid dosage form.
(vii) The storage conditions of solid dosage forms are not accurately managed to make drug available at 90% of its theoretical amount.

16.5 Chemical Kinetics Involved, Their Types, and Factors Affecting the Chemical Kinetics

16.5.1 Reaction Rate

The reaction rate can be referred as the speed at which the change in concentration of reactants into products over time [29].

$$\text{Rate} = \frac{(-\Delta[\text{Reactants}])}{\Delta t} = \frac{(\Delta[\text{products}])}{\Delta t}$$

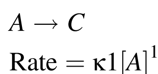
The above equation shows the association between reactants conc. and its rate and is known as rate law. The equation is represented as:

$$\text{Rate}\ \ \alpha[A]\chi[B]\chi$$
$$\text{Rate} = \kappa[A]\chi[B]\chi$$

Rate cannot be constant at any time "t"; it is a proportional concentration raised to some power.

16.5.2 Rate Law

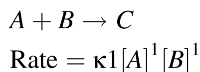
K is a rate constant, which solely depends on temperature in spite of concentration. With the help of rate constant κ, the rate of any concentration can be calculated. The exponent values present in reaction χ and are representation of reaction order; the overall sum of exponents is the representation of overall reaction order [30]. The overall reaction order is:



$$A \rightarrow C$$
$$\text{Rate} = \kappa 1[A]^1$$

Overall reaction order = 1.

Thus, it can say it is a first-order reaction:



$$A + B \rightarrow C$$
$$\text{Rate} = \kappa 1[A]^1[B]^1$$

Overall reaction order is 1 + 1 = 2.

Thus, it can say that the reaction is a second-order reaction. So, according to the order of reaction, there are three types of reaction kinetics that are known.

16.5.3 Apparent or Pseudo-Order Reaction

When concentration of one of the reactants is in large amount and it does not affect the overall reaction or its rate and it can be held constant, the reaction is called pseudo-order reaction. In any reaction in which hydrolysis occurs, the water concentration is in excess, so it is constant; however, it is a second-order reaction but acts like a first-order reaction, so it can be called as apparent or pseudo-order reaction [31].

16.5.4 Zero-Order Reactions

When the decomposition starts and continues at a constant rate and does not depend on concentration of reactants, this reaction is called as zero-order reaction [32].

$$\text{Rate law} = k_0$$
$$[A]_t = [A]_0 - k_0 t$$

$[A]$ t is the residual quantity of substance at time t.
K_0 is a zero-order rate constant.

Examples

Hydrolysis of aqueous drug suspension
Phytochemical degradation

16.5.5 First-Order Reaction

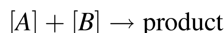
When the rate of reaction is dependent on one reactant, the reaction is called as first-order reaction [33].

$$\text{Rate} = k_1 [A]$$
$$\ln [A]_t = \ln [A]_0 - k_1 t \quad \text{Integrated form}$$

$[A]_0$ = initial amount of substance
K_1 = first-order rate constant

16.5.6 Second-Order Reactions

Second-order reaction can be explained in the manner when the speed of reaction is dependent on concentration of two reactants [34].



$$[A] + [B] \rightarrow \text{product}$$

When the initial concentrations of *A* and *B* are different, the following equation is achieved:

$$\text{Rate} = k_2[A][B]$$
$$\ln \frac{[B]_0}{[A]_0}\frac{[A]t}{[B]t} = k2t\left([A]_0 - [B]_0\right) \quad \text{Integrated form}$$

16.5.7 Chemical Kinetics

Chemical kinetics is the branch of physical chemistry, in which we study the rate of conversion of reactants into products [27]. Chemical kinetics also focus on the factors that affect reaction rate. This chemical reaction can be carried out by abiotic or biotic system.

The equation of the chemical kinetics is written as:

$$\text{Reaction rate} = \frac{\text{change in product concentration}}{\text{corresponding change in time}}$$

The main purpose of studying chemical kinetics is to understand the overall chemical change that occurs in the reaction. One major elementary step is known for conversion of reaction or reaction complex from single transition state to its chemical form. If elementary steps are more than one, then the reactants after passing through one intermediate state first convert into an intermediate product before conversion into final product. Figure 16.1 shows the formation of intermediate (I) by involving two reactants $k + 1$ and $k + 2$ and irreversibly converting into product $k + 2$. $k + 1$ and $k + 2$ are also designated as forward microscopic rate constant.

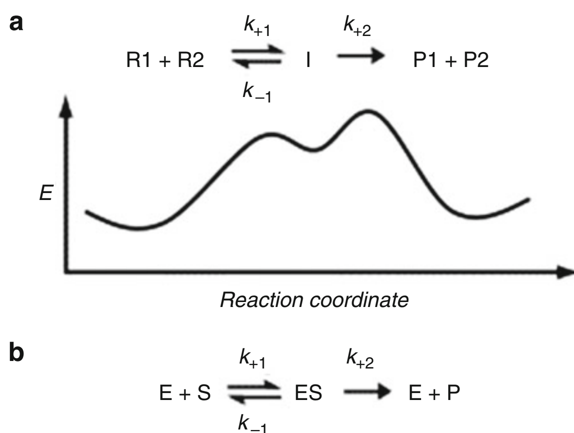


Fig. 16.1 Basic kinetic mechanism for conversion of two molecular reactants (R1 and R2) into their final product (P1 and P2) [28]

Another annotation of reverse rate ($k-1$) constant is also used if the given elementary step is reversible [28].

16.5.8 Types of Chemical Kinetics

Chemical reactions have been classified into further three groups, on the basis of rate of reactions.

16.5.8.1 Very Fast or Instantaneous Reaction

The reaction which is completed immediately at very fast rate is called very fast or instantaneous reaction. These reactions include ionic species and generally are ionic reactions. These reactions are completed in minimum time of 10–14 s.

16.5.8.2 Moderate Reaction

These reactions start and complete in a calculated time; these reactions occur in normal temperature. In these reactions, a large number of bonds in reactants break down, and many new bonds in products are formed. These reactions are generally molecular in nature.

16.5.8.3 Very Slow Reactions

These reactions occur in months and are very slow reactions; it requires a lot of time to complete the reaction. So, it is usually a quite difficult task to find the exact rate of reactions.

16.5.9 Factors Affecting Chemical Kinetics

There are four main factors that can affect rate of reactions; these include [35]:

16.5.9.1 Concentration of Reactants

The higher the number of reactants, the higher will be the rate of reaction by increasing the collision in a specific time period, but this condition is not applied for zero-order reaction. On the other hand, the higher concentration of products leads to a decrease in reaction rate.

16.5.9.2 Surface Area and Medium of Reactants

The reaction rate is also dependent on the surface area or medium of the reaction in which the reaction takes place; this condition is especially applied for the reactants, which are in heterogeneous phase. The rate of reaction may change according to the shape or size of the reactants in solid phase. Similarly, the variation in physical state of the reactants, vary the frequency of reaction, this state may be aqueous or organic, solid, liquid or gas, and polar or non-polar state.

16.5.9.3 Temperature

It is considered as a general rule for many chemical reactions that by increasing the temperature of the reaction medium will speed up the reaction. Therefore, the higher the temperature, the higher will be the average kinetic energy of the molecules and the higher will be the effective collisions in unit time as explained in Fig. 16.2. Still at some point, something change in chemical reaction may be reactants or chemical species that will stop or slow the chemical reaction.

16.5.9.4 Presence of a Catalyst

Catalyst can be explained as a substance that speeds up the reaction frequency without being used in a process. Catalyst has the capability of increasing the collisions between reactants and changes the pathway to obtain products, thus causing to generate more effective collisions. This results in decreased intramolecular bonding of reactants that leads to completion of reaction more quickly.

16.5.9.5 Pressure

The higher the pressure applied to reaction, the higher will be the rate of reaction. This factor is applied for gases but not significant for solids and liquids.

16.5.9.6 Mixing

Increasing the mixing of reactants will increase the speed of reaction.

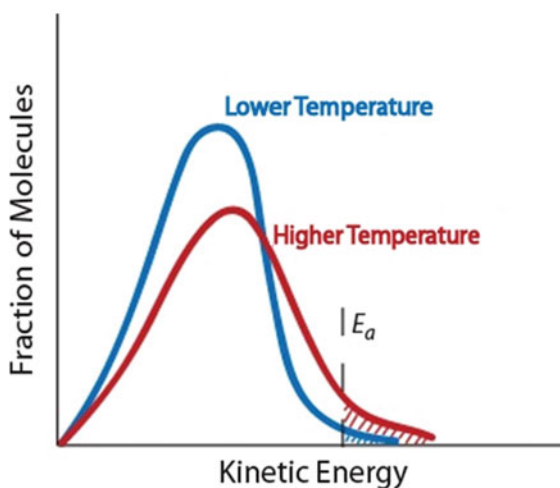


Fig. 16.2 Effect of temperature on the kinetic energy versus fraction of molecule [36]

16.6 Guidelines (Like FDA and ICH) for Stability Studies and Chemical Kinetics

16.6.1 General Guidelines for Stability Studies

General guidelines for stability studies are opted to assure the stability of formulations. There is a drug regulatory authority present in several countries, which enforce the drug manufacturers to submit the stability data regarding their product. The main objective to collect stability data from different manufacturers is to create uniformity in testing. The guidelines cover basic points that are linked with stability study. The first guidelines were present in the 1980s, which were later regulated by the International Conference on Harmonization (ICH) for registration of products [37].

16.6.2 Stress Testing

The stress testing process is applied to find out the degradable products in drug substance. Thus it can reveal the pathway of degradation and overall basic stability of formulation. The nature of stress testing entirely depends on its own drug mixture [39].

Stress testing is employed on an individual batch and used to evaluate the drug product at different conditions of temperature with 10° increment and humidity along with effect of oxidation, hydrolysis, photostability, and photolysis [40].

16.6.3 Climate Zone for Stability Testing

To assess the stability testing in pharmaceutical formulation, the world is distributed into four zones depending on the atmospheric conditions of the zone. These conditions are elicited by calculating the mean annual temperature and mean annual humidity of the climate. Thus, by calculating this temperature and humidity, stability testing conditions or long-term and accelerated testing conditions were derived.

Table 16.1 ICH and WHO guidelines for stability studies [38]

ICH guidelines	Titles
QIA (R2)	Stability testing of new drug releases and products
Q1B	Stability testing: photostability testing of new drug substances and products
Q1C	Stability testing for new dosage forms
Q1D	Bracketing and matrixing designs for stability testing of drug substances and products
Q1E	Evaluation of stability data
Q1F	Stability data package for registration application in climate zones III and IV

Table 16.2 Climate zone, along with countries and their mean partial water vapor pressure and long-term testing conditions [38]

Climatic zone	Climate/ definition	Major countries/ region	MAT*/mean annual partial water vapor pressure	Long-term testing conditions
I	Temperate	Europe, United Kingdom Russia USA	≤15 °C/≤11 hPa	21 °C/45% RH
II	Mediterranean and subtropical	Japan, Southern Europe	>15–22 °C/>11–18 hPa	25 °C/60% RH
III	Hot and dry	India	>22 °C/≤15 hPa	30 °C/35% RH
		Iraq		
IVa	Hot and humid	Iran	>22 °C/>15–27 hPa	30 °C/65% RH
		Egypt		
IVb	Hot and very humid	Brazil, Singapore	>22 °C/>27 hPa	30 °C/75% RH

Table 16.1 gives the standard environmental conditions as given by WHO. The stability conditions according to the climate were adjusted to make products and its manufacturing easy for industrial or practical use [41] (Table 16.2).

16.6.4 Selection of Batches

Stability studies are applied differently on different kind of batches. When the drug product is at initial stages and requires registration, stability studies are carried out on one batch, while for new product, three primary batches are required for stability studies. On the other hand, an established drug can provide data of even two stability studies. The overall quality of the drug formulation should explicit the overall quality prepared on production scale.

16.6.5 Container and Closure System

The testing on the product is done on container prescribed for that product, and appropriate closure is required for marketing of the product. The packaging of drug products should be blister packs, aluminum strip packs, HDPE bottles, etc. Secondary packs are also part of closures. The products found in different containers are tested independently, though the containers containing bulk of drug are tested in prototype container, if they are immediately processed for the actual packaging.

16.6.6 Specifications

Stability studies of different kind of drug substance such as degradable drugs or drugs that are susceptible to change due to storage conditions require a list of analytical processes and proposed criteria discussed in ICH, Q6B and Q6A. These criteria and processes are collaboratively called as specifications. These testing include physiochemical testing, biological testing, and microbiological testing.

16.6.7 Test Frequency

Test frequency is decided on the basis of the duration of storage period of drug substance to assure the stability studies. Every drug is proposed to test again after 12 months. The drugs, which have long-term storage conditions, are tested in every 3 months, 6 months, and annually for 1st year, 2nd year, and thereafter throughout the period, respectively.

16.6.8 Storage Conditions

The drug substance is tested for its storage conditions by applying thermal stability testing and its moisture-absorbing tendency and by measuring its potential for solvent loss. The storage studies selected those conditions or length, in which drug remains unchanged till its storage, shipment, and use (Table 16.3).

16.7 Why We Perform Stability Studies for Solid Dosage Forms

First and foremost, the need for stability study is that it is the legal requirement for the drug formulation. It assures the patients about the rationality of drug. Stability study of solid dosage form is an important and challenging task as it can affect active matter present, and it is mandatory even if the defined efficacy is known. Sometimes chemical degradation affects the drug ingredients which lead to the decrease in concentration of drug dosage form. The main purpose to carry out stability study is to acknowledge the quality of the drug product that varies due to different atmospheric conditions such as moisture, temperature, light, etc. There are many

Table 16.3 Storage conditions [43]

Study	Storage condition	Minimum time period covered by data at submission
Long term	25 °C ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ± 5% RH	12 months
Intermediate	30 °C ± 2 °C/65% RH ± 5% RH	6 months
Accelerated	40 °C ± 2 °C/75% RH ± 5% RH	6 months

other contributing factors involved in deformity of the formulation such as microbial contaminations, traces of metals, leaching process, excipients, shelf life, and recommended storage conditions.

16.8 Why We Study the Chemical Kinetics in Stability Studies of Solid Dosage Forms

Chemical kinetics is found to be important in determination of stability of pharmaceuticals ($t_{1/2}$) and the shelf life.

16.8.1 Half-Life

$t_{½}$ is also known as half-life which can be defined as the time required by drug for its decomposition or decay by 50%.

16.8.2 Shelf Life ($t_{0.9}$)

The time required by the drug or formulation to decay by 90% from its original concentration. The shelf life of drug can be calculated by [42]:

$$t_{90} = \frac{(a - 0.9a)}{k_0} = \frac{0.1a}{k_0}$$

where a is the initial concentration, k_0 specific rate constant for zero-order reaction, and t_{90} unit of time per concentration.

Shelf life of any drug or formulation indicates the time from the date of manufacturing and packaging until the maintenance of its therapeutic integrity; however, its physical character remains unchanged.

16.9 Pharmaceutical Instability Due to Variation in Chemical Kinetics

Pharmaceutical instability is generated by oxidation, isomerization, hydrolysis, photolysis, and epimerization in liquid, solid, and semi-solid products. The reactions involved and the environmental factors of solid dosage forms produce physical or chemical instability of pharmaceutical preparations [43]. The chemical kinetics and hydrolytic degradation of pharmaceutical preparation of ampicillin at 35 °C and at ionic strength of 0.5 were documented. The decomposition starts at a range of 0.8 to 10 pH which leads to first-order kinetics, and it occurred in both acid and base catalysis. It was seen that the instability of the product increases the chemical kinetics of the ampicillin by addition of sucrose [44].

16.10 Applications of Stability Studies, Chemical Kinetics, and Their Advantages and Disadvantages

16.10.1 Applications of Stability Studies

Pharmaceutical stability plays an important role in various aspects. It includes:

(i) Drug discovery and drug development as the unstable drug can deteriorate immediately.
(ii) Pharmaceutical stability is important to achieve acceptable drug concentration for desired pharmacological effect.
(iii) These studies assure the patients about the drug.
(iv) Stable drug can easily be evaluated for pharmacokinetic studies, as unstable drug degrades eventually even after taking blood samples from animals.
(v) Drug stability is an important factor for researchers for in vivo studies.
(vi) Pharmaceutical stability is very important for the screening of prodrugs.
(vii) Stability studies can find out the unwanted agents formed during chemical reaction of active ingredients.

16.10.2 Stability Issues

During stability studies, there are several problems which have to be faced during processing. These include:

(i) Physical stability, sedimentation, and compaction.
(ii) Processing requires care during handling.
(iii) Uniform drug delivery cannot be achieved sometimes.

16.10.3 Applications of Chemical Kinetic Studies

The chemical kinetics has the following advantages:

(i) It gives the evidence for the mechanism of chemical process occurring in the reaction mixture.
(ii) It gives information regarding the most effective way of occurring a chemical reaction.
(iii) It also gives the information regarding how quickly a reaction can reach up to equilibrium level.
(iv) It elaborates the individual steps and their detailed nature of the reaction.

16.11 Role of Pharmacist in Overall Stability Studies and Chemical Kinetics of Solid Dosage Forms

Preparation of pharmaceutical products is the major part of pharmacist's duties. Pharmacist plays a major role in setting the stability standards and also the chemical kinetics of solid dosage forms. Maintenance of stability studies must be part of the mission of pharmacist as defined in Good Manufacturing Practices [45].

(i) Pharmacist ensures stability study to predict the shelf life of solid dosage forms; it has been done by accelerating the reaction to decompose rapidly, by increasing temperature.
(ii) Pharmacist guaranteed the stability testing to maintain pharmaceutical product fresh and elegant till its shelf life.
(iii) Stability studies are also necessary for the disposal of active ingredients to the patient throughout its expected shelf life.
(iv) Pharmacist assures the uniformity of dosage, even when administered in multiple-dose container.
(v) Pharmacist should ensure the chemical and physical stability of active ingredients.
(vi) Pharmacist should keep an eye on the degradation route, physical state of matter, reaction kinetics, and other stabilization strategies.

16.12 Conclusion

The above stability and chemical kinetic studies give the knowledge about instability and chemical kinetic factors, their types and issues, and degradation reactions that fall in the category of chemical kinetics. Degradation reactions become the reason of chemical instability. The reactions involved due to chemical instability are oxidation, reduction, hydrolysis, photolysis, etc. Degradation studies in chemical kinetics are considered as an important aspect for the drug discovery regarding pre-formulation studies, for the purpose of achieving optimum storage conditions and stabilizing drug against degradation reactions and for the prediction of the shelf life of drug product. Thus the stability of drug formulation is an important factor for the achievement of drug product and its maintenance throughout its shelf life, availability of its active ingredients, and assurance of the uniformity of dose dispensed in multiple container.

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