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Oral Mucosal Drug Delivery and Therapy

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To my mother… I am unable to express my love and gratitude in words, so let me just say thank you for sacrificing so many precious moments of your own life to assure my happiness and success.

MJR

Canım anneme … Bugüne kadar bana verdiğin karşılıksız sevgi, özveri ve güvenle yaşamımı anlamlı kıldın, sonsuz sevgi ve teşekkürlerimle… SŞ

To my mother… you always gave me unconditional love, devotion and trust, which made my life momentous. Thank you with my love forever… SŞ

To my mother … you are the most loving and caring person I have known, and you always had complete trust and faith in your children – thank you.

IP

Preface

Academia and industry have expressed a high interest throughout the past three decades in delivering drugs to or across the oral mucosa for the purpose of achieving desired therapeutic outcomes. However, because the membranes that line the oral cavity exhibit relatively poor permeability to drugs, and due to the fact that only a limited number of drugs possess the innate physicochemical properties to allow them to inherently cross the mucosa in clinically relevant amounts, we have failed to witness a large number of oral mucosal drug delivery systems become commercially available. This situation has stimulated much interest in conducting research that has focused on increasing the potential drug candidate list for oral mucosal therapeutic applications. Research on the use of oral mucosal permeation enhancers and mucoadhesives has resulted in advances in our knowledge of how to modify drug permeation through the oral mucosa and delivery system retention at the site of administration.

It is the editors' belief that the prospect of the oral cavity as a site for drug delivery has yet to meet its full potential and that the oral mucosal route of administration is ideally suited to improve the delivery of several existing drugs. Such delivery systems would offer market differentiation for these drugs through improved, pain-free, patient-friendly delivery systems that, when optimized, would offer a definite therapeutic improvement over existing treatments. However, great challenges face formulators who aim to deliver drugs locally to the membranes that line the oral cavity or systemically across the oral mucosa. Such challenges require innovative solutions to create drug delivery systems that provide a convenient, patient-acceptable means to relieve clinical symptoms and include ingredients that manipulate the bioavailability of drugs across the oral mucosa or provide prolonged retention at the site of absorption. This volume examines the area of oral mucosal drug delivery and the therapeutic opportunities for the use of the oral mucosa as a site of administration for drug delivery. It is our hope that the contents of this book will arm future researchers with the relevant information for them to develop new drug delivery systems that result in the oral mucosa becoming an important future site of administration for drug delivery.

In Chap. 1, Thomas P. Johnston reviews the most relevant aspects of oral mucosal and mouth anatomy and physiology and relates its relevance to local and

systemic oral transmucosal drug delivery. Some of the concepts addressed involve the advantages and disadvantages of oral mucosal drug delivery, the various sites of drug delivery within the mouth, factors that influence drug delivery associated with the gross and the microenvironment within the oral cavity (e.g., mucus, saliva, and salivary glands), and practical considerations regarding tissue irritation and/or damage when using this route of drug administration. Johnston also examines the role of permeation enhancers and buffering agents/pH modifiers in oral transmucosal drug delivery. The fundamental anatomical and physiological information provided in this chapter will build a sound background for those pharmaceutical scientists directly involved with the formulation of dosage forms intended for oral mucosal drug delivery.

In Chap. 2, Rathbone, Pather, and Şenel explore the reasons for developing oral mucosal drug delivery systems, and identify the key considerations in the design and development of oral mucosal drug delivery systems. Throughout the chapter, the authors describe the characteristics of many of the delivery systems that have been successfully developed and commercialized for use in this site for drug delivery.

The permeability of many drugs through the mucosa of the oral cavity is slow due to the inherent barrier properties of the mucosa that line the oral cavity. Therefore, the enhancement of permeation of the drug is needed to extend the drug candidate list for this route of administration. In Chap. 3, Pather and Kolli examine the use of chemicals that promote the passage of the drug through the oral mucosa and describe the different classes of compounds that may be useful to enhance oral mucosal drug permeation. Pather and Kolli adopt a broad view of the concept of "chemical methods for enhancing delivery," thus, effervescent agents and chemicals that assist in retaining the dosage form on the mucosa for an extended time, thereby allowing a longer time for drug permeation, are included in this chapter.

In Chap. 4, Sandri et al. examine the mechanism of action, functional characteristics, selection, and assessment of mucoadhesive polymers as enabling excipients for oral mucosal drug delivery. The authors identify and discuss several classes of polymers that have been proved to possess pronounced adhesion properties when placed in contact with oral mucosa. They also define the key properties that facilitate these molecules achieving prolonged adhesion onto oral mucosa, which include their ability to spread over the mucosal surface, their swelling properties, their ionic (cationic and anionic) charge density, and their hydration and consequent mucus dehydration properties. The chapter provides an in-depth look at the assessment of the mucoadhesive properties of the compounds and the rationale for their selection by the formulation scientist for their inclusion in an oral mucosal drug delivery system.

Authors Giannola, De Caro, and Sutera examine the physical methods for enhancing oral mucosal delivery in Chap. 5. The authors examine the use of sonophoresis, iontophoresis, and electroporation methods in the area of oral mucosal drug delivery and review the ability of these techniques to increase the drug flux through the oral mucosal membranes. The authors highlight that these physical methods are extensively used to enhance drug permeation through the skin but have yet to become widely used for increasing drug permeation of the membranes that line

the oral cavity, despite the fact that they are very promising in this regard and are gaining in popularity due to their noninvasive and convenient means for local or systemic delivery of drugs.

In Chap. 6, Kolli and Pather define the methods used to characterize oral mucosal drug delivery from the perspective of both the drug and its formulation**.** The authors highlight that even though drug delivery across the oral mucosa has emerged as a useful alternative for compounds that cannot be delivered orally, standardized methods to evaluate drug absorption across oral mucosal membranes, either in vitro or in vivo, and standardized techniques used to characterize oral mucosal drug delivery systems have yet to be agreed upon. Their chapter provides a comprehensive review of the current in vitro and in vivo methodologies employed in the literature for evaluating oral transmucosal absorption of compounds. In addition, it reviews the use of buccal cell cultures as a means to study oral mucosal drug absorption. In the second part of their chapter, the authors examine of the methods used to test oral mucosal drug formulations including residence time, mucoadhesion and drug release.

In Chap. 7, Rathbone, Pather, and Şenel take an in-depth look at systemic controlled release oral mucosal drug delivery systems and the clinical opportunities that currently exist for this type of drug product. The chapter describes the potential of the oral cavity as a site for the systemic delivery of drugs alongside some of the problems and their solutions and examines the research in these areas and how they have resulted in extending the clinical opportunities for the use of the oral mucosa as a site for drug delivery.

Tablets for systemic oral mucosal drug delivery are reviewed in Chap. 8 by Rane and Moe. The chapter focuses on the formulation and performance of solid dosage forms commonly used in oral transmucosal delivery. The authors highlight the specific challenges associated with the oral cavity as a route of drug administration together with the products used for transmucosal delivery that are more effective and sometimes safer than conventional dosage forms. They also discuss clinical studies that directly compare conventional dosage forms with oral transmucosally delivered products. The authors expertly define the basic principles of oral transmucosal drug delivery and explore new developments in-depth. Examples of formulation technologies and clinical performance from successful and widely known oral transmucosally delivered products are provided. Overall, this chapter comes to the conclusion that there is a large scope in further development of strategies for oral transmucosal drug delivery that could be applied to as yet unexplored molecules.

A relatively new area of research and application of the oral cavity is that of the formulation of delivery systems for photosensitisers that are therapeutically used in oral cavity photodynamic therapy. Photodynamic therapy is a clinical treatment that combines the effects of visible light irradiation with subsequent biochemical events that arise from the presence of a photosensitizing drug to cause destruction of selected cells. Following administration, the photosensitizer accumulates in the target cells and a measured light dose of appropriate wavelength is then used to irradiate the target tissue that activates the drug. In Chap. 9, Donnelly reviews the current status and future potential of this area to oral mucosal drug delivery. The

chapter provides a clear message that photodynamic therapy has an important role to play in the treatment of neoplastic and dysplastic disease at body sites amenable to irradiation, and in the future this may include the oral cavity where local delivery can have a large role to play in the treatment of such local oral mucosal diseases.

In Chap. 10, Caramella et al. describe the current status of medical devices as a supportive care for oromucosal pathologies. The chapter examines the opportunities offered by medical devices and provides an interesting example, even though there are currently many unmet needs in the treatment of oromucosal pathologies. The authors introduce the area that includes definitions and relevant regulations and the oral conditions that can be treated with a medical device. In addition, the mechanisms of action by which medical devices function are reviewed, and a list of products available on the market is included in the chapter. At the end of the chapter, the authors summarize their ongoing work in this area and provide a fascinating case study on a new improved class II medical device.

In the final chapter, Hughes and Ghosh provide a general overview of the U.S. Food and Drug Administration's regulatory considerations for intraoral drug product development and marketing approval. The authors highlight that effective drug delivery through the oral mucosa is complex, and only a few products have so far achieved commercial success. They discuss the often unpredictable scientific hurdles and suggest that a good understanding of the regulatory requirements for product development is critical for maximizing resources and positive interactions with the regulatory authorities. The chapter provides an overview that will allow scientists to successfully navigate through the U.S. regulatory approval process and underscores that such an attempt will require an interdisciplinary approach from the legal, clinical, chemistry, clinical pharmacology, nonclinical and biopharmaceutics perspectives.

The editors of this volume sincerely thank the authors for their time, efforts and patience in the compilation of this volume. We are indebted to their exceptional knowledge and understanding of the area and for their willingness to put down in words and share their years of experience in this field. We have enjoyed the opportunity to compile the book, and we hope that other scientists will benefit from reading the authoritative chapters contained within this volume.

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Indiran Pather serves as Director of Research and Professor of Pharmaceutics at the California Northstate University College of Pharmacy (CNUCOP). After completing the B. Pharm. degree, he practiced as a pharmacist in South Africa. He subsequently completed the M. Pharm. and D. Pharm. degrees in Pharmaceutics. He taught in South Africa before joining the University of Missouri as a Research Assistant Professor. Since then, he has worked in the industry and held positions of increasing responsibility up to Director of Pharmaceutical Sciences. At CNUCOP, he teaches courses in Compounding and Biopharmaceutics and Drug Delivery. His research has involved analytical chemistry, novel drug delivery systems, and the formulation of cosmetics, pharmaceuticals, and animal health products. Prof. Pather holds 17 U.S. patents and has published 31 patent applications. He is a reviewer for several international pharmaceutical journals. His present research interests involve the formulation of novel drug delivery systems (especially oral transmucosal), finding new uses for established drugs, and in natural products. He currently serves as consultant to pharmaceutical companies.

Michael J. Rathbone is Founder and Managing Director of ULTI Pharmaceuticals, a veterinary pharmaceutical company located in New Zealand. He was formerly Professor of Pharmaceutical Technology and Dean, School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia. Prior to this, he was Associate Professor of Pharmaceutics, School of Pharmacy, Griffith University, Australia, and the Director of Research and General Manager of InterAg, where he spearheaded the companies veterinary controlled drug delivery research and directed their national and global collaborative research activities. Dr. Rathbone obtained his PhD in Pharmaceutics from the University of Aston, Birmingham, UK. He is a Fellow of the Controlled Release Society and has edited eight books in the area of modified release drug delivery and 10 special theme issues of journals.

Sevda Şenel is Professor of Pharmaceutical Technology at Hacettepe University, Faculty of Pharmacy, Turkey. She is internationally recognized for her contribution to the areas of mucosal drug and vaccine delivery, veterinary drug delivery and dental drug delivery. Prof. Şenel has been the recipient of many national and

international grants and has published more than 150 communications to scientific meetings, papers in refereed journals, book chapters and books. She has served as the Editor-in Chief of the *FABAD Journal of Pharmaceutical Sciences* and currently serves on the Editorial Boards of the *Journal of Drug Delivery Science and Technology, Pharmaceutical Technology and Development*, and the Polish Chitin Society Journal, *Progress in the Chemistry and Application of Chitin and its Derivatives*. She served as the President of the European Chitin Society for two terms and currently sits on several committees of the Controlled Release Society and on two advisory committees at The Drug and Medical Device Agency of Turkey. Professor Şenel has received many honors, awards, and fellowships, and most notably has been honored with the Distinguished Scientist Award by the Academy of Science of the Turkish Pharmacists Association and the Hacettepe University Science Award.

Chapter 1
Anatomy and Physiology of the Oral Mucosa

Thomas P. Johnston

1.1 Introduction

1.1.1 Background and Rationale of Oral Mucosal Drug Delivery

The oral route of drug administration still continues to be the most popular route for the administration of medications. This is true for a number of reasons, some of which include: (a) ease of administration, (b) patient compliance, (c) ease of preparation of oral dosage forms (tablets, capsules, solutions, etc.), and (d) the ability to ingest a suitable volume of liquid (typically water) to effectuate rapid and complete dissolution of a solid dosage form. However, there are a number of reasons that other routes of drug administration are preferable. An alternative route of drug administration, which provides several distinct advantages relative to the oral route of drug administration, is by diffusion into and through oral mucosae located in the oral cavity. Specifically, two mucosae located in the oral cavity, namely the sublingual and buccal mucosa, have been the most widely studied [1,2]. Drug delivery through either mucosa presents several distinct advantages. For example, absorption of a medication through either the sublingual or buccal mucosa limits the degree of enzymatic and/or acid-catalyzed degradation of the drug substance that could potentially occur following oral administration [1]. This is especially pertinent to newer biologically active macromolecules, i.e., therapeutic proteins and peptides, which typically exhibit greater pharmacological activity compared to traditional low molecular weight organic drug compounds, and which are exquisitely

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sensitive to changes in pH, ionic strength, and hydrodynamic conditions (air/water interface, stirring, etc.). Additionally, absorption through the sublingual or buccal tissue avoids "first-pass" extraction in the liver, since a drug absorbed from the oral cavity eventually enters the internal jugular vein and, subsequently, the systemic circulation [3]. Thus, for drug substances that are highly cleared by the liver, absorption by the mucosae found in the oral cavity offers a distinct therapeutic advantage. Lastly, if ingestion of food or medication by the oral route has been restricted (i.e., nothing by mouth, NPO) due to a particular medical condition, then drug administration across the mucosae in the oral cavity provides an alternative route of drug administration that does not involve venipuncture (an invasive procedure). For example, the oral mucosal route may be useful for patients who have recently undergone surgery, or have experienced upper gastrointestinal tract disease that would affect the oral absorption of a drug.

The first drawback to the administration of drug compounds by the oral mucosae is that the medication should preferably be highly potent. While this is not an absolute prerequisite for oral mucosal drug delivery, there are practical considerations that make this a preferred drug property. For instance, there is only a window of 5–6 h for drug administration by the oral mucosa due to patients behaviors (i.e., resumption of eating, drinking, etc.). Second, the oral mucosae do not contain microvilli similar to the gastrointestinal tract, and therefore, the absorptive surface area is limited. Finally, the thicknesses of the absorbing membranes in the oral cavity are greater than the epithelium of the gastrointestinal tract. For these reasons, there has been an increased emphasis to utilize oral mucosal drug delivery for highly potent therapeutic agents; for example, protein and peptide drugs. Historically, parenteral administration has been the most common route for protein and peptide drug delivery. However, delivery of therapeutic proteins and peptides by the parenteral route can sometimes be associated with infections and pain upon repeated administration, which leads to poor patient compliance. Additionally, protein and peptide drugs administered by the gastrointestinal route exhibit poor oral bioavailability due to gastric acid hydrolysis and subsequent inactivation as well as extensive gut and/or hepatic clearance. Thus, noninvasive mucosal routes of drug administration have been explored as an alternative to systemic drug delivery for this class of compounds.

The transdermal route is restricted to potent, lipophilic compounds. Moreover, it does not provide rapid blood levels, and is less permeable than the oral mucosa. Thus, various absorptive mucosae, including nasal, ocular, pulmonary, rectal, vaginal, buccal, and sublingual, have been investigated for systemic delivery. As mentioned above, the buccal and sublingual routes of drug administration, being convenient and easily accessible sites, would appear attractive alternative routes of drug administration for both systemic and local drug delivery. This chapter aims to provide basic information concerning the anatomy and physiology of the oral mucosa and its relevance to local and systemic drug delivery for both traditional low molecular weight organic drug molecules and biologically active therapeutic macromolecules.

1.1.2 Advantages of Oral Mucosal Drug Delivery

The buccal mucosa is highly perfused with blood vessels and offers a greater permeability than the skin. This is easily seen in Table 1.1, which lists mean blood flow rates to various tissues. Relative to the nasal and rectal routes of drug administration, the buccal mucosa has low enzymatic activity and, therefore, causes comparatively lower drug inactivation. As an example, ease of administration and excellent accessibility to buccal mucosa makes application of the dosage form painless, site-specific, and allows for prolonged delivery and easy removal from the application site. The presence of a pair of buccal mucosae in the oral cavity permits the application of a drug delivery system at different sites, either on the same mucosa or, alternatively, on the left or right buccal mucosa. This is particularly advantageous if the delivery system contains a drug or excipient that mildly and reversibly damages or irritates the mucosa.

Buccal and sublingual drug delivery systems can be designed to allow unidirectional drug release so that it can be protected from the local environment of the oral cavity. Since there is no "first-pass effect," a substantial reduction in dose can be achieved, thereby reducing dose-related side effects. Buccal or sublingual administration also permits the inclusion of a permeation enhancer or protease inhibitor or pH modifier in the formulation to modulate the mucosal environment at or near the application site to improve the drug's bioavailability. Any systemic toxicity of these enhancers, inhibitors, or pH modifiers is reduced, because potential mucosal irritation is limited to a well-defined area. The buccal mucosa, in particular, is well suited for modifications in the formulation because it is less susceptible to irreversible damage. In the event of toxicity, the delivery of drugs can be terminated by simply removing the formulation from the absorbing mucosa.

Table 1.1 Blood flow to various regions of the oral mucosa in the rhesus monkey[a]

Region/tissue	Mean blood flow (mL/min/100 g tissue)
Dorsal surface of the tongue	100.6
Lip	49.9
Buccal mucosa	20.3
Attached gingiva[b]	19.5
Ventral surface of the tongue	13.9
Floor of the mouth	12.2
Soft palate	9.2
Hard palate[b]	7.0

[a] Values from reference [25]
[b] Keratinized region

1.1.3 Disadvantages of Oral Mucosal Drug Delivery

Drugs which are unstable at buccal pH, irritate the oral mucosa, have a bitter or unpleasant taste, odor, and cause allergenicity may not be suitable for administration by this route. The surface area available for buccal absorption (about 50 and 27 cm^2 in humans for buccal and sublingual mucosa, respectively) is much smaller than the gastrointestinal, nasal, rectal, and vaginal mucosae. The buccal mucosa is continuously bathed by saliva, and the secreted saliva lowers drug concentration at the absorbing membrane. These two factors (surface area and drug concentration), along with the permeability coefficient of the drug, affect the overall absorption rate. Also, overhydration of the buccal mucosa may form a slippery surface and disrupt the structural integrity of the formulation by causing swelling of any bioadhesive polymers that may be included in the dosage form. In contrast, patients may secrete too little saliva ("dry mouth syndrome"), which may cause impaired dissolution of the active agent and, thus, delayed absorption [1]. It should also be noted that the buccal mucosa is less permeable than any of the mucosae discussed above, with an average thickness of 500–600 μm. In contrast, the average thickness of sublingual mucosa is approximately 100–200 μm. Involuntary swallowing of saliva containing dissolved drug or swallowing the delivery system itself would lead to major drug loss from the site of absorption. Talking, eating, and drinking affect the retention of the delivery system and, therefore, may constitute limitations associated with the buccal route of drug administration. In addition, there is a risk of choking from a dislodged drug delivery device.

1.2 Anatomy and Physiology of the Oral Mucosa

The oral mucosa is anatomically divided into three layers (Fig. 1.1): the outermost layer of stratified squamous epithelium, followed by basement membrane and, lastly, the connective tissue composed of the lamina propria and submucosa [4]**.** The permeability of buccal mucosa is 4–4000 times greater than the skin epidermis and less than that of the intestinal mucosa. In the oral cavity, the order of permeability is sublingual > buccal > palatal. This is due to the physical characteristics of each tissue, with sublingual tissue being relatively thin and nonkeratinized, while palatal tissue is keratinized. The permeability barriers of the oral mucosa are described below.

1.2.1 Epithelium

The buccal epithelium, as an example, consists of approximately 40–50 layers of stratified squamous epithelial cells. The basal layer is mitotically active and produces epithelial cells, which then migrate through a number of intermediate layers.

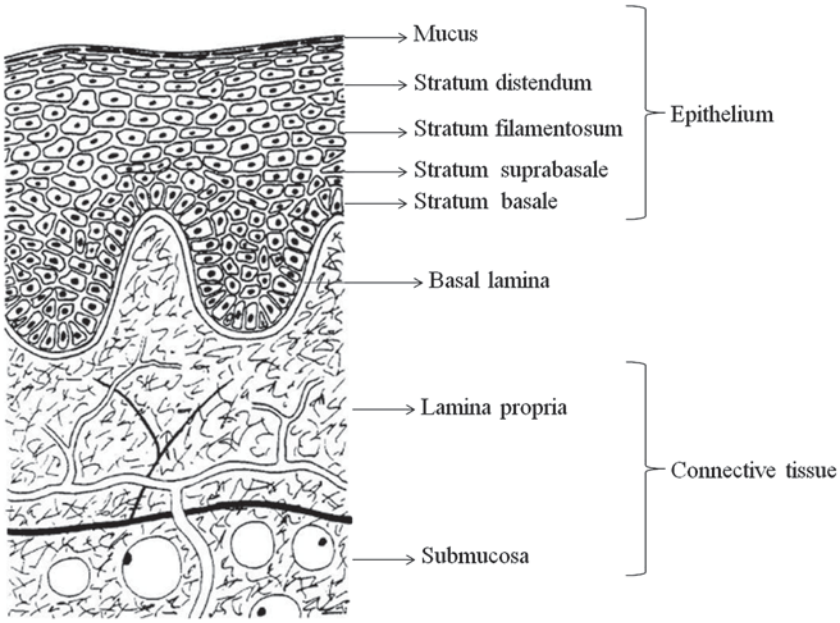

Fig. 1.1 Cross section showing the principal components of buccal mucosa. (Source: From Ref [4])

As the cells migrate to the surface, they increase in size and become flattened. Cytoplasmic organelles disappear and protein content is elevated. Cells are then shed at the surface of the epithelium. The turnover time for the epithelium is typically considered to be 5–6 days.

Composition of the epithelium varies with location in the oral cavity. The gingiva and hard palate (areas subject to mechanical stress) are keratinized, whereas the soft palate and sublingual regions are nonkeratinized. Thus, the thickness varies from 500 to 800 μm. One important biochemical feature of the buccal epithelium is the presence of large molecular weight (40–70 kDa) proteins called tonofilaments. The epithelial cells are surrounded by a matrix rich in carbohydrate–protein complexes, which act as a lubricant to promote cell-to-cell adhesion. The buccal epithelium is also characterized by the presence of large intercellular junctions, primarily gap junctions.

The superficial layer of epithelium is the predominant barrier to drug diffusion. Permeation studies with horseradish peroxidase [5] and lanthanum nitrate [6] have shown that the flattened superficial layers constitute the major barrier, while the lower layers are relatively permeable. This is true in both keratinized and nonkeratinized epithelium, suggesting that keratinization does not offer as much resistance to buccal drug permeation as once thought. The permeability barrier of the epithelium is mainly due to the presence of membrane-coating granules (MCGs), which are described below.

1.2.2 Membrane-Coating Granules

MCGs are spherical or oval-shaped organelles, 100–300 nm in diameter. The MCGs contain primary lipids and accumulate when the cells leave the basal layer, begin to differentiate, and migrate toward the surface. They are present in both keratinized and nonkeratinized epithelium, but differ in composition. The granules fuse with the cell membrane at the superficial layers and discharge their contents into the intercellular spaces. MCGs themselves, or their discharged contents, typically influence the permeability of the epithelium to permeants.

1.2.3 Basement Membrane

The basement membrane is the boundary between the basal layer of the epithelium and the connective tissues. It is a trilaminar structure consisting of lamina lucida (upper amorphous layer), lamina densa, and a sublayer of fibrous material. The lamina densa is composed of highly ordered collagen, which imparts strength to the structure. The basement membrane appears as ridges and folds that project into the epithelium. Thus, it has a larger surface area compared to the epithelium and, as such, this larger surface area may present a moderate degree of resistance to drug permeation/transport by affecting the diffusional pathlength of the permeant (drug molecule). The basement membrane serves three important functions: adherence between the epithelium and the underlying connective tissue, mechanical support to the epithelium, and a barrier to the passage of cells and macromolecules.

1.2.4 Connective Tissues

Connective tissue consists of lamina propria and submucosa, if present. The lamina propria is a continuous sheet of connective tissue comprised of collagen, elastic fibers, and cellular components. It is insufficiently dense to offer resistance to relatively large molecules. However, the hydrated matrix in this tissue promotes the passage of hydrophilic permeants. The lamina propria is rich in blood vessels that open into the internal jugular vein, thus avoiding first-pass metabolism.

1.3 Types of Mucosa in the Oral Cavity

There are three different types of oral mucosa present in the oral cavity: masticatory mucosa, specialized mucosa, and lining mucosa. Each type of mucosa is located in a different region in the oral cavity, and has unique characteristics. In addition, the three types of mucosa differ in their relative surface area in the oral cavity.

Masticatory mucosa covers the gingival region as well as the hard palate. These regions are subject to abrasions and shearing that are associated with the mastication process. Accordingly, the masticatory mucosa is keratinized and usually tightly attached to the underlying structure, e.g., bone. In some regions, the attachment of the masticatory mucosa is directly to the underlying structure, and there is no submucosa present. The masticatory mucosa comprises approximately 25 % of the surface area in the oral cavity [3]. Specialized mucosa is found on the dorsum of the tongue, specifically in the taste bud regions. The epithelial layer found here is both keratinized and nonkeratinized. Specialized mucosa is very tightly bound to the underlying muscle of the tongue. Specialized mucosa comprises approximately 15 % of the surface area of the oral cavity [3]. Lining mucosa covers the remaining regions of the oral cavity, and accounts for approximately 60 % of the surface area in the oral cavity [3]. The lining mucosa is an elastic surface capable of stretching with ordinary movements such as speech and mastication [7]. The epithelial layer of the lining mucosa is nonkeratinized and shows considerable variation in thickness depending on what region of the oral cavity it is located. The lining mucosa is loosely attached to the underlying structures by connective tissue. The lining mucosa is also more permeable than the other types of oral mucosa.

1.4 Drug Penetration Across the Oral Mucosa

The rate and extent of drug absorption are influenced by the permeability of the buccal and sublingual mucosa (membrane factors), physicochemical properties of the drug (permeant factors), and other environmental factors. But, prior to examining each of these factors in detail, a brief review will be provided on the routes of drug transport across the oral mucosa. The buccal mucosa has been arbitrarily selected when discussing the routes of drug delivery through the oral mucosa.

1.4.1 Routes of Drug Delivery

As discussed previously, the superficial layer of the oral mucosa is composed of a layer of stratified epithelial tissue. The epithelial cell membranes are essentially lipophilic, but the spaces between the cells are basically hydrophilic. Thus, the oral epithelium may be thought of as an area composed of lipophilic and hydrophilic regions. As a result, there are essentially two routes for drug transport across the oral mucosa: the paracellular route and the transcellular route [8]. Although drugs are able to use both routes simultaneously, one route will be preferred over the other due to the inherent properties of the drug/permeant (discussed below).

The paracellular route refers to passage of a drug through the space surrounding the individual epithelial cells. As noted above, the area between the epithelial cells is essentially hydrophilic in nature, although there may be lipophilic components

secreted into this space. Because this area is hydrophilic, this is the preferred transport route for drugs that are hydrophilic in nature and have a molecular weight of less than approximately 500 Da. In contrast, the paracellular route is a transport barrier for drugs that are lipophilic in nature primarily because the solubility of a lipophilic compound in a hydrophilic environment is low.

The transcellular route refers to passage of a drug through the cells of the epithelial layer. The epithelial cell membranes are lipophilic, and so transport through the transcellular pathway is favored for lipophilic compounds. It should be noted that the intracellular space, e.g., the cytoplasm, is hydrophilic. However, the main resistance to drug transport by the transcellular route is the cellular membrane, and so transport across the intracellular space (cytoplasm) is relatively rapid and does not constitute a significant transport barrier [9]. Transcellular transport is rapid for lipophilic compounds for a variety of reasons. For example, the relative surface area for the transcellular route is significantly larger than that for the paracellular route [9]. In addition, the pathlength for a drug to cross the oral mucosa by transcellular transport is much shorter than for paracellular transport [9].

Transcellular absorption of drugs is mediated by passive diffusion. This is particularly true for low molecular weight lipophilic drugs. In essence, passive diffusion involves the transport of a drug from an area of high concentration to an area of low concentration. In the context of drug delivery by the oral mucosa, the area of high concentration is the oral cavity, whereas the area of low concentration is the blood supply. The low concentration gradient of the blood supply is maintained by normal blood flow, which continually carries absorbed drug away from the oral mucosa and results in the maintenance of "sink conditions."

Passive diffusion follows Fick's laws of diffusion, which state that the absorption rate is proportional to the drug concentration and the surface area available for drug absorption. However, there are other important considerations that affect the rate of diffusion, such as the ionization state of the drug molecule. In particular, the ionization state of a drug is important for drug diffusion through the transcellular route, because non-ionized drugs exhibit greater lipid solubility as compared to the ionized form. For this reason, the rate of passive diffusion through the transcellular route is related to the amount of drug that exists in the non-ionized form. Drug ionization state is, of course, related to the pK_a of the individual drug, as well as the pH of the local environment as described by the Henderson–Hasselbalch equation. These concepts will be more fully described below when buffering agents/pH modifiers are discussed.

1.4.2 Permeant Factors

Permeation of a compound across the buccal mucosa is mainly dependent on molecular size, lipophilicity, and ionization. Small molecules (<100 Da) are rapidly transported through the buccal mucosa. As the molecular size increases, permeability typically diminishes for hydrophilic substances. For unionized molecules, as the

lipophilicity rises, permeability typically increases. For many ionizable drugs, maximum permeation occurs at a pH where the drug compound remains primarily unionized. Thus, assuming a saliva pH of approximately 7.2, as an example, either weakly basic drug compounds that have pK_a values less than or equal to 7.2, or weakly acidic drugs that have pK_a values greater than or equal to 7.2 would ensure that a large percentage (≥50%) of the ionizable drug molecule existed in the unionized form.

1.4.3 Environmental Factors

Environmental factors such as mucus, saliva, salivary glands, and movement of the oral tissues may also decrease the rate and extent of drug absorption across buccal mucosa.

1.4.4 Mucus

Mucus is the intercellular ground matrix consisting of mucins and inorganic salts, which surrounds epithelial cells. It serves as a lubricant, thus facilitating movement of cells relative to one another. At buccal pH, mucous molecules join together to form a gelatinous layer over the epithelial cells with a thickness varying from about 40 to 300 μm. It plays a major role in the bioadhesion of drug delivery system [10].

1.4.5 Saliva and Salivary Glands

The secretion from major and minor salivary glands is known as saliva. It is primarily composed of water (99%), with a pH around 6.5–7.5 [11]. This pH plays an important role in the passive diffusion of unionized drugs. The normal resting (unstimulated) flow rate of saliva is approximately 100–500 μl/min, with a total volume of saliva in the oral cavity being approximately 1 mL [12]. Others report a daily saliva production rate of 1.0–1.5 L, which would correspond to a saliva production rate of approximately 700–1000 μl/min [13]. An increase in salivary secretion (due to mechanical pressure, chemicals in the diet, anticipation of a meal, etc.) makes it difficult for a delivery system to be retained for a significant amount of time at the absorption site. Similarly, any retentive system placed adjacent to the salivary gland may result in rapid dissolution/erosion of the delivery system.

1.4.6 Movement of the Oral Tissues

Swallowing, talking, and eating may lead to dislodgement of the device in the oral cavity. Movement of the tissues is less when individuals are sleeping, but

swallowing does occur during sleep. Typically, when the volume of saliva in the oral cavity reaches about 1.1 mL, an involuntary swallowing reflex is triggered, and the saliva is subsequently swallowed. The movement of the tongue may also influence the delivery of drugs from a mucoadhesive retentive system, owing to compression of the dosage form against the hard palate by the tongue, induction of suction pressure, and a general sweeping action of the tongue across the dosage form and tissues [14].

1.4.7 Tissue Irritation Resulting from Oral Transmucosal Drug Delivery

This chapter would be incomplete without mentioning the changes to tissue properties that can potentially arise when various pharmaceutical additives are incorporated into dosage forms/formulations utilized in oral transmucosal drug delivery. While mucoadhesive polymers, enzyme inhibitors, pH modifiers, and permeation enhancers are just several examples of additives routinely used in dosage forms for oral transmucosal drug delivery, the discussion here will be limited to permeation enhancers and pH modifiers only. This is because enzyme inhibitors are generally reserved for peptidic compounds administered by the oral mucosae. Additionally, mucoadhesive polymers represent another class of dosage forms aimed at achieving retention of the dosage form on the mucosal surface for protracted delivery of the incorporated drug molecule. In general, irritation of the oral mucosal tissue is very subjective and may differ widely from treatment to control subjects. Most tissue irritation occurs as a result of penetration/permeation enhancers. Occasionally, tissue irritation may occur if a pH modifier drastically changes the pH of the microenvironment surrounding the dosage form for an extended period of time. Evaluation of toxicity and irritation is necessary as it relates to: (1) mucosal tissue irritation, (2) extent of damage to the mucosal cells, and (3) rate of recovery.

1.4.8 Mucosal Tissue Irritation

Tissue irritation is a complex phenomenon involving interaction among the solution properties of the vehicle, mucosal transport, biological transport, and local drug disposition. To date, no definite relationship has been established between the structure of a penetration enhancer and the degree of irritation it may cause following buccal or sublingual application. However, a relationship between the pK_a value of an ionizable compound (benzoic acid derivatives) and irritation, as measured by the degree of erythema, has been previously reported [15, 16]. In general, the most effective penetration enhancers would induce the maximum degree of irritation to mucosal tissues. Preferably, this irritation would be quickly reversible with no permanent alteration to the structure and function of the cells that line the oral cavity.

1.4.9 Extent of Damage to Mucosal Cells

Permeation enhancement implies possible alteration of the protective permeability barrier either by: (1) an increase in the fluidity of intercellular lipids (a relatively nontoxic strategy) and/or (2) extraction of intercellular lipids or denaturation of cellular proteins (much more damaging/toxic to tissue and cells). Therefore, it is imperative that the permeation/penetration enhancer: (1) exert a reversible effect, (2) not be systemically absorbed, and (3) not cause cumulative toxicity or permanent changes in the barrier properties. The literature is replete with individual examples of pharmaceutical additives (permeation enhancers) that have caused varying degrees of tissue irritation and, therefore, will not be discussed here.

1.4.10 Methods Used to Evaluate Membrane Damage

Numerous methods have been developed over the years to assess the degree of damage to biological membranes induced by various permeation enhancers. Examples of methods utilized to evaluate irritation to tissues either during or following oral transmucosal drug delivery include, but are not limited to, the following: (1) morphological examination of the tissue by scanning or transmission electron microscopy, (2) morphological examination of the tissue with light microscopy and appropriate staining, e.g., hematoxylin and eosin (H&E), (3) determination of the extent of hemolysis caused by a permeation enhancer, (4) determination of the release of cellular constituents, e.g., lactate dehydrogenase (LDH), (5) measurements of the changes in the electrical resistance of the membrane (temperature corrected transepithelial electrical resistance (TEER) measurement), and (6) measurements of the changes in the permeability to various markers, e.g., inulin, mannitol, and fluorescein isothiocyanate (FITC)-dextran. The important criteria for a pharmaceutical additive that functions as a permeation enhancer is whether or not the enhancer induces a permanent or reversible change in the mucosal membrane properties.

1.4.11 Rate of Recovery of Mucosal Membranes

The rate of recovery is, in general, inversely related to the extent of membrane damage. A greater and more rapid recovery is observed from permeation enhancers that induce minimal damage, such as acylcarnitines [17] and sodium glycocholate, compared to permeation enhancers such as sodium deoxycholate [18] and polyoxyethylene-9-lauryl ether. The permeability of the tight junction is sensitive to the extracellular calcium concentration. Reclosing of tight junctions has been shown to be accelerated if there is a high extracellular calcium concentration, rather than an elevated cytoplasmic calcium concentration [19]. But, as mentioned above, there are very few tight junctions in normal oral mucosae. In general, cell turnover

is quite rapid (days) in the oral cavity, so most minor abrasions or irritations result in only temporary and mild discomfort to the patient.

1.4.12 Miscellaneous Toxicity Concerns

Additional toxicity concerns include interference with normal metabolism and function of mucosal cells, e.g., water absorption by mucosal cells [20]. The unconjugated bile acids are known to block amino acid metabolism and glucose transport [21]. There is also a possibility of biotransformation of these enhancers to toxic or carcinogenic substances by hepatic monooxygenases. Absorption of permeation enhancers into the systemic circulation can also cause toxicity, e.g., azone and hexamethylene lauramide [22], which are absorbed across the skin. Moreover, changes in membrane fluidity may alter the activity of membrane-bound transport proteins and enzymes. Thus, the judicious selection of permeation enhancers is a requisite for the formulation of drugs intended for oral transmucosal drug delivery. Ideally, formulations intended for oral transmucosal drug delivery should not include permeation/penetration enhancers so as to avoid any irritation or damage to the cells that comprise the oral mucosae.

1.4.13 Permeation Enhancers

Permeation enhancers are known to act by different mechanisms, which include increasing cell membrane fluidity, extracting structural intercellular and/or intracellular lipids, and altering cellular proteins or mucus structure and rheology. However, the selection of the permeation enhancer depends on the physicochemical properties of the drug, the site of administration in the oral cavity, and the nature of the vehicle and other excipients contained in the formulation. Permeation enhancers, which open tight junctions, are of little benefit in oral mucosal drug delivery, because tight junctions are uncommon in these tissues. In general, permeation enhancers should be safe and nontoxic, pharmacologically and chemically inert, nonirritating, and nonallergenic. Because of the structural differences in buccal mucosa, the use of traditional penetration/permeation enhancers that have been successfully incorporated in transdermal or intestinal drug delivery systems may find limited use in the oral cavity. It should be mentioned that because the sublingual mucosa is about one fifth as thick as buccal mucosa, a permeation enhancer is generally not required for enhancing sublingual drug absorption. Nevertheless, permeation enhancers used to improve drug absorption across other absorptive mucosae, may find limited use to improve drug penetration through buccal mucosa. It should be emphasized that the exact chemical structure/absorption enhancement activity relationships have not been completely characterized for permeation enhancers. As such, those permeation enhancers that have proven effective have been empirically identified through extensive in vitro cell culture and in vivo preclinical animal testing.

1.4.14 Buffering Agents/pH Modifiers

Chemical compounds that temporarily modify the pH of the microenvironment (the saliva/mucosa interface) are sometimes incorporated into formulations designed for oral transmucosal drug delivery. In general, these agents are typically weak acids and weak bases that do not drastically alter the pH of the microenvironment. A significant change in pH would obviously injure or irritate the underlying mucosal tissue, which is an undesirable effect. The inclusion of a pH-modifying agent is generally employed when a slight shift in the pH is required to help facilitate absorption of a weak base or weak acid drug. Typically, since the water-soluble salt form of a weak acid or base is incorporated into a dosage form, the problem of aqueous solubility in saliva is not a major limitation. However, if the water solubility of a weak base, as an example, is too low, the free base may precipitate from solution. While precipitation of the free base form of the drug from the aqueous saliva does not necessarily terminate drug absorption, it does significantly slow down the process of absorption, because in order for the drug to be absorbed, the free base must dissolve (undergo dissolution) in the saliva. This can potentially result in the dissolution of the free base in saliva as being the rate-limiting step in drug absorption. Theoretically, it would be desirable to have an ionizable drug's pK_a value in close proximity to the pH of the oral cavity, which is continuously bathed with saliva, such that a very slight increase or decrease in the pH has the potential to change the fraction of the ionizable drug that exists in the nonionized and ionized forms. The relationship that relates the solution pH and the pK_a of the ionizable drug to the fractions of the drug that exist in the nonionized and ionized forms is the Henderson–Hasselbalch equation [23].

Pharmaceutical formulators often include buffering agents into formulations to maintain the pH within a very limited range so as to intentionally cause a change in the proportions of the nonionized or ionized species of the weak base or weak acid drug. As an example, if a weak base drug molecule having a $pK_a = 6.8$ was incorporated into a dosage form for oral transmucosal drug delivery, and the pH of the saliva was assumed to be 7.2 (saliva pH normally varies between 6.5 and 7.5), one might desire more of the weak base drug to reside in the nonionized form to facilitate permeation through a lipoidal membrane. Using the Henderson–Hasselbalch equation, one could incorporate a mild buffering agent/pH modifier into the formulation such that the pH of the microenvironment would be increased to pH = 7.7 (approximately 0.5 pH unit above the assumed prevailing pH of 7.2), thus assuring that the released weak base would have approximately 88.8 and 11.2 % of the drug in the nonionized and ionized fractions, respectively. This would increase the concentration of the nonionized species available for transcellular permeation by approximately 17 % due to inclusion of the pH modifier when compared to a formulation without the pH modifier, and which had equilibrated with the saliva assumed to be at pH = 7.2 (nonionized = 71.5 %; ionized = 28.5 %). Increasing the concentration of the basic drug that exists in the nonionized form would increase the absorption rate according to Fick's first law of diffusion [24]. Thus, the use of pH

modifiers in oral transmucosal formulations find use to enhance drug absorption for drugs that are either weak acids or weak bases.

1.5 Conclusions and Future Directions

In recent years, there has been explosive growth in our understanding of the mechanisms associated with the absorption of drugs across the oral mucosae. Scientists from a variety of disciplines continue to elucidate the variables associated with the optimal formulation for oral transmucosal drug delivery to take advantage of this route of drug administration for drugs that exhibit a high "first-pass" effect due to intestinal and/or hepatic extraction, are subject to either extensive degradation by gastric acid or gastrointestinal enzymes, or would otherwise not be administered due to a patient's disease state (malabsorption syndrome, immediately postabdominal surgery, etc.). With the development of the different formulation approaches, the obstacles for the efficient delivery of most conventional low molecular weight drugs, as well as newer peptide and protein therapeutics, are slowly being overcome. However, methods to increase drug flux using permeation enhancers with minimal/negligible associated toxicity, strategies to inactivate proteolytic enzymes responsible for the degradation of therapeutic peptides and proteins, and innovative approaches with regard to controlled drug delivery require further investigation.

It is suggested that future research in the area of oral transmucosal drug delivery be focused on providing a highly potent, lipophilic (high $P_{o/w}$) drug molecule over a limited time window of 5–6 h without the requirement of penetration/permeation enhancers that would result in undue, irreversible damage to the oral epithelium, since the transmucosal absorption of the chemically based permeation enhancer is undesirable. New physical mechanisms for enhancing drug transport that would augment the primary mechanism by which drugs are absorbed across the oral epithelium (passive diffusion) should be investigated (e.g., sonophoresis, ionophoresis, electroporation, etc.).

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Chapter 2
Overview of Oral Mucosal Delivery

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2.1 Introduction

The oral cavity is an attractive site for the delivery of drugs either locally or directly into the systemic circulation. Its attractiveness resides in the fact that the mucosal membranes, upon which drug delivery systems are located, are readily accessible to patients or their carers. This means that the delivery technology can be precisely placed on the specific oral cavity membrane that is chosen as the site of absorption. It also means that the delivery system can be removed in order to terminate delivery if signs of adverse reactions are observed during treatment. The oral cavity represents a challenging area to develop an effective drug delivery technology. This arises due to the various inherent functions of the oral cavity (eating, swallowing, speaking, chewing), as well as the presence of the fluid that is involved in all these activities, saliva. This fluid is continually secreted into, and then removed from, the mouth. There are many advantages and disadvantages associated with the oral cavity as a site for drug delivery. Overall, however, it remains a viable option as a route for drug administration and has been extensively studied for that purpose [1–7].

In this chapter, the rationale behind companies pursuing the goal of developing oral mucosal drug delivery systems as well as the key considerations in the design and development of a drug delivery system intended for use in the oral cavity will be discussed. Finally, this chapter will briefly describe some of the formulation aspects of delivery systems that have been successfully developed to deliver drugs via this route.

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2.2 Reasons for Developing Oral Mucosal Drug Delivery Systems

Table 2.1 lists the advantages, while Table 2.2 lists the disadvantages of the oral cavity as a site for drug delivery. These tables have been constructed from information adapted from published reviews written by the present authors [1–7]. A perusal of these tables suggests that there are as many disadvantages as there are advantages for using the oral cavity as a site for drug delivery. Table 2.1 shows that the oral cavity is an attractive site for the delivery of drugs either locally or directly into the systemic circulation. Ultimately, the decision to utilize the oral cavity as a site for drug delivery should be based on a comparison to other sites of delivery with regard to the following parameters: the clinical objectives of the treatment, the inherent physicochemical properties of the drug, the relative advantages of the route, product differentiation opportunities, the patient population, the cost of production and R&D time.

2.3 Key Considerations in the Design and Development of Oral Mucosal Drug Delivery Systems

2.3.1 Influence of Oral Cavity Anatomy and Physiology on Drug Delivery

The anatomy and physiology of the oral cavity have a direct influence on the design of oral mucosal drug delivery systems. The anatomy and physiology have been comprehensively dealt with in a separate chapter of this book. This section provides a summary of the positive and negative influences of the various physiological and anatomical features of the oral cavity that may influence oral mucosal drug delivery system design and evaluation. These are compiled in Table 2.3.

2.3.2 Drug Absorption Across the Oral Mucosa

Two major routes of absorption are involved in oral mucosal drug permeation: the transcellular or intracellular route (where drugs permeate directly through the cells) and the paracellular or intercellular route (where drugs permeate by passive diffusion through the spaces between the cells) [11]. The paracellular route is favoured especially by hydrophilic drugs such as peptides/proteins which dissolve more readily in the aqueous fluids filling the intercellular spaces.

An example of a drug known to penetrate via the transcellular pathway is fentanyl [12], which is a highly lipophilic drug, whereas an example of a drug absorbed via the paracellular route is caffeine [13], which is a water-soluble drug.

Table 2.1 Advantages of the oral cavity as a site for drug delivery

Aspect	Advantage	Comment
Accessibility	The different sites in the oral cavity are easily accessible	This property increases patient convenience. Furthermore, the precise placement of the delivery system at any site in the oral cavity allows the targeting of a specific membrane, thus differentiating the different oral cavity routes
Administration	The ease of accessibility referred to above means oral mucosal drug delivery systems are easy to administer	This property increases patient acceptability for oral mucosal drug delivery systems
Removal	The ease of administration is matched by the ease of removal	Useful property in the event of adverse reactions. Delivery can be easily terminated if side effects from the drug are observed
Patient acceptability	Highly acceptable site for drug delivery by the patient	This site for drug delivery is well accepted by the patient, increasing patient compliance
First-pass effect	The oral mucosa is a well vascularized tissue and the blood vessels drain directly into the jugular vein [8]	Drugs penetrating the epithelium are delivered directly into the systemic circulation, thus avoiding the hepatic first-pass effect
Avoidance of gastrointestinal tract environment	Drugs absorbed across oral mucosa directly into the systemic circulation	Direct absorption of drugs into the systemic circulation avoids hydrolysis in the gastrointestinal tract. Swallowing of dissolved drug should be avoided
Enzymatic barriers	The buccal mucosa provides an environment with reduced peptidase and protease activity	Significantly less drug metabolism is seen in the oral cavity
Cellular turnover time	The cellular turnover time of the oral mucosa is estimated to be 4–14 days [9]. This is intermediate between the slow turnover rate of the skin and the fast gastrointestinal rate	A mucoadhesive device may be worn for many hours or even days without disturbing its adhesion due to rapid cell division. In addition, fairly rapid recovery is possible if slight tissue damage occurs due to wearing a dosage form [7]
Microenvironment	The microenvironment of a dosage form placed in the oral cavity can directly and easily be modified	The physicochemical conditions in a small volume of saliva that bathes the oral mucosa at the site of administration can be changed with minimal adverse effects

Table 2.2 Disadvantages of the oral cavity as a site for drug delivery

Aspect	Disadvantage	Comment
Membrane permeability	In general, oral cavity mucosa shows low permeability to drugs	Membrane thickness varies from a few hundred micrometres for the sublingual region to 500 μm for the buccal mucosa
Surface area	The surface area of the oral cavity is small; it is approximately 214 cm^2 [10]	The oral cavity has a smaller absorptive surface area compared to the small intestines
Saliva	Saliva is continually secreted into the oral cavity from major and minor salivary glands	The continuous secretion of saliva (0.5–2 L/day) can lead to dilution of the drug or excessively fast erosion of a dosage form. For patients secreting too little saliva (dry mouth syndrome), there may be insufficient saliva to allow dissolution of the drug
Swallowing	Salivation leads to swallowing which effectively removes drug from the target site of absorption	Efficacy of the drug would change and side effects would increase
Taste receptors	The tongue contains taste receptors that may present difficulties to patients and decrease compliance with drugs that are bitter	This problem may be greater with certain patient populations such as the young and the elderly
Membrane flexibility	Some of the oral mucosa (e.g. sublingual and buccal mucosa) is flexible and flexes as a consequence of normal functions of the mouth (e.g. speaking, chewing or swallowing). This may adversely affect the dosage form	If the oral mucosal drug delivery system contains a mucoadhesive, movements of the mouth or tongue may dislodge the dosage form from the site of administration
Choking hazard	Involuntarily swallowing of the delivery system could lead to choking	This potential hazard should be considered during the design of the delivery system and evaluated during the research or development phase
Inconvenience	A buccal delivery system may cause inconvenience to the patient when they are eating or drinking	If food or liquid consumption occurs post application of, say, a mucoadhesive dosage form, the temperature and pH of the consumed material may affect drug release
Tissue irritation	For some drugs, tissue irritation may arise following the use of an oral mucosal drug delivery system	Irritation may lead to faster absorption and/or pain at the site of application
Drug candidates	Drug candidate list is small	The list of drugs that can be incorporated into oral mucosal drug delivery systems is limited due to low permeability of the mucosa which results in low bioavailability, and the small drug loading capacity of the delivery systems. Also, salivation and swallowing remove drug from the absorption site

Table 2.3 The influence of physiological and anatomical features of the oral cavity on drug delivery system design and evaluation [1–7]

Aspect	Advantage	Disadvantage
Saliva	Promotes dissolution of drug	Constant secretion and removal by swallowing can cause drug and delivery system to be removed from the intended site of absorption
	Wets dosage forms containing mucoadhesives, thereby promoting adhesion to the oral mucosa	
	Saliva continually bathes the surface of the oral mucosa and maintains a moist, stable environment	Saliva is a relatively mobile fluid
	Compared to the secretions of the gastrointestinal tract, saliva contains less mucin, limited enzymatic activity and virtually no proteases	
Flexible membrane	Some membranes are less flexible than others (e.g. gums, hard palate)	Can cause delivery systems to dislodge from the mucosa
Structures (teeth, gums, tongue, cheek, and palate)	Provide a variety of sites for drug delivery	The taste receptors in the tongue can prevent the formulation of bitter drugs; taste masking is difficult since pleasant tastes increase salivation and drug swallowing
	Delivery systems or devices can be affixed to structures, including non-absorbing structures such as teeth	
pH	Saliva has a slightly acidic pH which is favourable for a wide range of drugs	
	pH can by modified easily at the site of administration	
Keratinized mucosa	Usually located in regions of the mouth that do not flex (gum, palate)	Provides an additional barrier to drug absorption
Non-keratinized mucosa	More permeable than keratinized mucosa (buccal, sublingual)	Tend to be in regions of the mouth that are flexible
Membrane thickness	Sublingual mucosa is relatively thin, therefore this region is good for the purpose of rapid drug absorption	Buccal mucosa is relatively thick and absorption may be too slow to be useful for drug delivery
Surface area	Generally sufficient to allow for drug absorption of drugs with appropriate physicochemical properties	Relatively small compared to other absorption sites of the body
Eating		Can cause dislodgment of delivery systems
Drinking		Drinking can cause excessive dissolution or erosion of the delivery system
		Hot or acidic fluids can change the rate of drug release, or alter the mucoadhesive properties of the dosage form

Table 2.3 (continued)

Aspect	Advantage	Disadvantage
Swallowing		Removal of drug and/or dosage form from the intended site of absorption
Mastication	Chewing can distribute drugs around the oral cavity, increasing the surface area for absorption. Useful property for chewing gum delivery systems	Chewing can cause the patient to bite into the delivery system

Table 2.4 Desirable drug physicochemical properties for formulation of an oral mucosal drug delivery system [3–6, 15]

Formulation considerations	Ideal limits
Aqueous solubility	> 1 mg/mL
Lipophilicity	10 < oil:water partition coefficient < 1000
Molecular weight	< 500 Da
Melting point	< 200°C
pH of saturated aqueous solution	pH 5–9
Required dose deliverable	< 10 mg/day
Irritation potential, which is the net effect of many physicochemical properties	No irritation to buccal tissue

In addition to these major pathways, other transport mechanisms (e.g. carrier-mediated transport) play a role in the transport of some drugs across the oral mucosa [14].

2.3.3 Influence of Drug Properties on Oral Mucosal Drug Delivery

The physicochemical properties of the drug play a crucial role in the design and formulation of an oral mucosal drug delivery system. It is of paramount importance that the physicochemical properties of the drug are characterized in order to allow for initial selection and subsequent formulation into an oral mucosal drug delivery system. The physicochemical properties of the drug that need to be known prior to its formulation into an oral mucosal drug delivery system are shown in Table 2.4.

2.3.4 Facilitation of Drug Effectiveness from an Oral Mucosal Drug Delivery System

Two factors influence the effectiveness of drug delivery from a delivery system designed for use in the oral cavity. The first is time of retention of the drug delivery

system in contact with the oral mucosa; the second is the permeation rate of the drug across the oral mucosa.

The ability to retain the drug delivery system in contact with the oral mucosa at a particular location can be achieved through the incorporation of carefully selected mucoadhesive polymers into the formulation. This results in the delivery system having an intimate contact with the oral mucosa for a prolonged time. When mucoadhesive polymers rapidly and securely interact with the mucin molecules, found on the surface of the oral mucosa, it results in intimate contact of the dosage form with the mucosa. The prolonged contact time allows for a longer duration for absorption of the drug. It also reduces the pathway for diffusion of released drug between the surface of the delivery system and the surface of the mucosa.

Increasing the permeability of the drug through the oral mucosa is another approach [16] used to assure therapeutic levels of a drug via the buccal route. This is commonly achieved through the use of a penetration enhancer in the formulation. Various chemicals have been used as permeation enhancers. These include surfactants, bile salts, fatty acids and non-surfactants (such as cyclodextrins, chitosan and Azones) [6,17,18,19–21].

Mucoadhesive polymers [22–24] and penetration enhancers used for oral mucosal delivery have been extensively reviewed by several authors in recent years [16,25] and readers are referred to these reviews for further information.

2.4 Oral Mucosal Drug Delivery Systems

A recently published review suggested that oral mucosal drug delivery systems are actively being conceptualized and invented, and significant time is being devoted in both academia and industry to research this route of drug delivery [7]. The same review highlights that there are less than 50 registered products available for buccal/sublingual delivery in the USA at its time of writing (2012) [26]. Not many active pharmaceutical ingredients (APIs) have successfully reached the marketplace as drugs for oral transmucosal delivery. Some of these, such as nicotine and nitroglycerine, have been used in buccal/sublingual delivery dosage forms for many years. There is, thus, a disparity between the intense research activity over the past two decades and the products for oral mucosal drug delivery actually reaching the market [7]. A number of oral mucosal drug delivery systems such as tablets, lozenges, sprays, wafers, strips, films, etc. have been described in the literature [27–29]. Some of these are briefly reviewed below.

Abstral This is a sublingual tablet comprising a rapidly disintegrating, fast-acting formulation of fentanyl citrate. Abstral was the first approved fast-acting, rapidly disintegrating tablet formulation for breakthrough cancer pain in the USA (other formulations were non-disintegrating). The product is now marketed by ProStrakan Ltd. across the principal European markets [30]. Abstral is formulated in six strengths: 100, 200, 300, 400, 600 and 800 µg, distinguishable by the shape of the tablet (round-, oval-, triangle-, diamond—"D"—and capsule-shaped, respectively) and by debossing on the tablet surface.

Actiq This product is a lozenge containing 200, 400, 600, 800, 1200 or 1600 µg of fentanyl citrate now marketed by Teva Pharmaceuticals Industries Ltd.'s Cephalon unit. It was the first product labelled for breakthrough cancer pain (1998). The lozenge is formulated into the shape of a lollipop. Thus, it can be described as a solid formulation (lozenge) on a plastic stick (an integral oromucosal applicator). The lozenge dissolves slowly in the mouth for absorption across the *buccal mucosa*. An Actiq lozenge is formulated as a white to off-white compressed powder drug matrix attached using edible glue to a fracture-resistant, radio-opaque plastic applicator, marked with the dosage strength. Its formulation includes dextrates (equivalent to approximately 2 g of glucose), sucrose (approximately 30 mg confectioner's sugar) and propylene glycol (part of the artificial berry flavour and imprinting ink) as excipients. Actiq should be placed in the mouth against the cheek and should be moved around the mouth using the applicator, with the aim of maximizing the amount of mucosal exposure to the product. The Actiq lollipop should be sucked, not chewed, as absorption of fentanyl via the buccal mucosa is rapid in comparison with systemic absorption via the gastrointestinal tract. The patient should consume the lollipop over 15 min.

Aftach Nagai [31] was among the first to pioneer the bioadhesive tablet drug delivery system in the early 1980s. The first product developed by him contains a steroidal, anti-inflammatory, triamcinolone acetonide, and is still on the market for the treatment of aphthous stomatitis (AFTACH; Teijin Pharma, Japan) [32].

Breakyl The first product to be approved in the European Union, using the Bio-Erodible MucoAdhesive (BEMA) drug delivery technology is Breakyl. It consists of a small, bioerodible polymer film for application to the buccal mucosal membranes (inner lining of the cheek) (BioDelivery Sciences International, Inc.) [33]. BEMA films were designed to rapidly deliver a drug dose across the mucous membranes for time-sensitive conditions, or to facilitate administration of drugs with poor oral absorption.

Buccastem This is a tablet formulation of prochlorperazine used for the treatment of nausea and vomiting, marketed by Alliance Pharmaceuticals Ltd. [34]. The tablet is placed in the buccal area where it releases the drug over a few hours. Each buccal tablet contains 3.0 mg prochlorperazine maleate. When the tablets are placed in the buccal cavity, they form a gel from which the prochlorperazine is released and absorbed. The plasma levels achieved at steady state on a dosage regimen of one buccal tablet twice daily are similar to those observed with the standard oral dosage of one 5 mg tablet taken three times daily. Its formulation contains compressible sugar, Povidone K30, xanthan gum, locust bean gum, talc, magnesium stearate and riboflavin sodium phosphate.

Buccolam This oral mucosal solution, containing 10 mg midazolam in 2 mL, is for paediatric use [35]. Buccolam (ViroPharma SPRL) is provided in a prefilled, age-specific dose formulation for administration to an area between the cheek and gum. It is a clear, colourless solution with a pH between 2.9 and 3.7. Its formulation contains sodium chloride, water for injections, hydrochloric acid for pH adjust-

ment and conversion of midazolam to the hydrochloride salt and sodium hydroxide (again for pH adjustment).

Epistatus This liquid buccal formulation of midazolam is available for the treatment of status epilepticus and serious tonic–clonic seizures in community settings. It is an unlicensed medicine made under Manufacturers Specials licence MS 123. Compared to its competitor, rectal diazepam, it provides a more convenient-to-use, and less embarrassing, option for the child.

Fentanyl Oralet This was the first Food and Drug Administration (FDA)-approved (1996) formulation developed to take advantage of oral transmucosal absorption for the painless administration of an opioid in a formulation acceptable to children.

Fentora Effervescence is used in this buccal tablet to promote the absorption of fentanyl. It became the second fentanyl oral transmucosal dosage form to be commercially marketed (Teva Pharmaceuticals Industries Ltd.'s, Cephalon unit) with an indication for breakthrough cancer pain [36,37]. The effervescent delivery system exhibits a much higher bioavailability than the same dose of fentanyl from an Actiq lollipop. The tablet is placed in the buccal cavity (above a premolar, between the gum and the cheek) where it disintegrates over approximately 10 min, thereby releasing the drug. The fentanyl buccal tablet received approval from the European Commission in 2008 under the trade name, Effentora.

Intermezzo This is a sublingual tablet containing zolpidem tartrate that is available in two strengths (3.5 and 1.75 mg) for the indication of middle-of-the-night insomnia [38,39]. It is made by Transcept Pharmaceuticals Inc. of Point Richmond. The formulation is designed to enhance sublingual permeation. The product is unique in that females are recommended a lower dose.

Loramyc (Oravig) This mucoadhesive tablet containing 50 mg of miconazole is based on the Lauriad™ technology. The inactive ingredients are milk protein concentrate, hypromellose 2208, maize starch, lactose monohydrate, sodium laurylsulphate, magnesium stearate and talc. The tablet gradually becomes hydrated and sticks to the proteins of the mucous surface, and then releases the active pharmaceutical ingredient on a prolonged basis [40]. They are white to slightly yellow tablets with a rounded side and a flat side debossed with "L." Loramyc is applied to the upper gum just above the incisor tooth. The rounded side of the tablet should be applied to the upper gum by holding the tablet in place for 30 s with a slight pressure of the finger over the upper lip. With each application of the mucoadhesive tablet, the tablet should be applied to alternate sides of the upper gum.

MetControl A metformin medicinal chewing gum developed by Generex Biotechnology Corporation for the treatment of type 2 diabetes mellitus and obesity is available commercially [41]. The smaller dose of buccally administered drug is expected to reduce the gastrointestinal irritation and bloating caused by metformin.

Oral-lyn A liquid formulation of regular recombinant human insulin that is delivered to the buccal mucosa is available. It uses the RapidMist technology which supplies a fine mist of the formulation to the mouth. Insulin absorption is limited to the

mouth with no entry into the lungs. This technology uses the formation of microfine, thin membrane, mixed micelles made from the combination of insulin and specific absorption enhancers that encapsulate and protect the insulin molecules. Oral-lyn buccal insulin spray has been shown to produce a significant reduction of HbA1c compared with a control group, with no adverse events [42].

Sativex This is an oromucosal spray containing delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), medicines derived from cannabis, to be delivered to multiple sclerosis (MS) patients suffering from muscle spasticity [43]. It is a yellow/brown solution in a spray where each millilitre contains 38–44 and 35–42 mg, respectively, of two extracts from *Cannabis sativa* L., folium cum flore (*Cannabis* leaf and flower). The extracts are in the form of soft extracts, corresponding to 27 mg delta-9-tetrahydrocannabinol and 25 mg cannabidiol. It is recommended that the spray should be directed at different sites on the oromucosal surface each time the product is used.

Striant This buccal system is designed to adhere to the gum or inner cheek. It provides a novel treatment option for the 4–5million men who require testosterone replacement therapy for a deficiency or absence of endogenous testosterone associated with hypogonadism [44].

Subutex and Suboxone Tablets These are tablet formulations, made by Reckitt Benckiser Pharmaceuticals Inc. and contain buprenorphine for initiating treatment of opioid dependence. Subutex contains only buprenorphine hydrochloride. This formulation was developed as the initial product. The second medication, Suboxone contains naloxone to guard against misuse [45]. Subutex is available as 2 mg sublingual tablets. The tablet usually fully dissolves under the tongue within 5–10 min. It contains monohydrated lactose, mannitol, maize starch, Povidone K30, citric acid and sodium citrate as excipients. Suboxone tablet contains 8 mg buprenorphine (as hydrochloride) and 2 mg naloxone (as hydrochloride dihydrate) or 2 mg buprenorphine (as hydrochloride) and 0.5 mg naloxone (as hydrochloride dihydrate). Its excipients are similar to those listed under Subutex tablets.

Suboxone Film This is a fast-dissolving sublingual film containing buprenorphine and naloxone in which the PharmFilm® technology is utilized. This technology is a strip dosage form [46] claiming to be absorbed via the buccal or sublingual mucosa. In general, the thin strip dosage form has an area of usually no more than about 15 cm^2 (and often much less) and a thickness of 0.2 mm at the maximum. Although larger films are possible, they are less pleasant to use and the convenience of portability is compromised. Hence, a disadvantage of thin strips is that they have a low dose-loading capacity (no more than about 40 mg/strip at most). Suboxone films have replaced Suboxone tablets in order to better control misuse and accidental paediatric exposure. The individually packaged films are more difficult for children to open [47].

Subsys Available as a sublingually administered single-dose spray formulation of fentanyl in a novel delivery device, Subsys offers numerous benefits to patients who

experience episodes of breakthrough cancer pain and recently received approval by the FDA [48].

Triaminic and Theraflu These thin strip series were formulated for the cough and cold market. They were claimed to be the first products of this type with a systemically absorbed drug. These over-the-counter products showed that the thin strip technology may be used for local effects in the mouth and throat; or it may contain a drug that is released in the oral cavity, swallowed and then absorbed in the gastrointestinal tract.

2.5 Concluding Remarks

The oral cavity remains an attractive site for drug delivery and the future potential of oral mucosal drug delivery looks favourable. Several commercially successful delivery technologies have been developed for oral mucosal application. It is envisaged that in the future, oral mucosal drug delivery systems will provide the platform for the successful delivery of more drugs. It is attractive for the delivery of biologics but problems of poor permeation of large molecules must be overcome.

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Chapter 3
Chemical Methods for Enhancing Oral Mucosal Delivery

Indiran Pather and Chandra Sekhar Kolli

3.1 Introduction

The oral cavity has been used for systemic drug delivery, especially for those drugs that may be destroyed by the harsh conditions prevailing in the gastrointestinal tract, or for those drugs that are extensively metabolized during their passage through the gastrointestinal mucosa, and by the liver. The unfavorable conditions that are encountered include low pH and the presence of enzymes. By direct drug absorption into the circulatory system, these risks are avoided. Drug delivery via the oral mucosal route has become more popular in recent times partly due to a greater need for alternate delivery mechanisms for very sensitive drugs, and partly as a consequence of the financial successes of some products, notably Actiq and Fentora. The former factor has assumed much greater prominence recently with respect to research studies, due to the rapid development of peptide therapeutics.

Some drugs are absorbed relatively well through the oral mucosa (e.g., fentanyl) while others are not sufficiently well absorbed to deliver the required dose via this route. In the latter case, the drug would have no utility unless its absorption was increased to an extent that provided desirable blood levels. In the former case, it may still be desirable to enhance the absorption of the drug to improve the efficacy and the therapeutic response. For example, it may be desirable to increase the initial rate of absorption for a quicker onset of action. This is exemplified very well by the case of fentanyl [1], where the drug naturally permeates the oral mucosal tissues, but enhancement of this effect decreases the time to onset of relief of breakthrough cancer pain. Thus, enhancement of drug delivery is important both in the case where

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the unenhanced drug would be too slowly absorbed to be effective and, also, in the case where absorption occurs at a reasonable rate absent in any pharmaceutical intervention. Drug absorption may be enhanced by physical methods (see Chap. 5) or by chemical methods as described here.

A chemical that enhances the rate of permeation of biologically active substances across the mucosa is highly desirable in many instances and such chemicals have been extensively studied. There are, also, several chemicals which do not increase the permeation rate but, by other mechanisms, increase the amount of the drug that is absorbed and they do so to an extent that a useful formulation can be developed. Some of these chemicals serve to hold a dosage form in place, thus allowing a longer period for drug absorption to occur. Since the rate of permeation is not increased, this latter group of compounds should not be referred to as "permeation enhancers" but can be described, more generally, as mucoadhesives. In many instances, drug absorption is inherently too slow to be of practical value and, in the absence of a mechanism which keeps the dosage form in close contact with the mucosa, salivary flow would carry the drug away from the preferred absorption site in the oral cavity. From this perspective, mucoadhesives could be considered chemical substances that improve the absorption of drugs.

Some substances inhibit the effect of naturally occurring enzymes that metabolize drugs. Since more of the drug is available for absorption, such substances would, also, enhance absorption. It should be emphasized that mucoadhesion and metabolism inhibitors only improve delivery of drugs that have an inherent ability to permeate the mucosa to a reasonable extent; in the absence of such ability, these types of additives would have little impact on drug delivery.

If rapid absorption, resembling that of a subcutaneous injection, can be attained without the use of needles, it is a major advantage in the delivery of many drugs. Likewise, if sustained, slow absorption can be achieved over the course of several hours, it may be favorable to the action of certain other drugs. Rapid absorption, reduced breakdown, and sustained delivery of the drug can all be achieved by the addition of chemical additives to the basic formulation, and this chapter discusses these concepts. The enhancement of absorption may be direct, as occurs in the action of permeation enhancers, or indirect, for example, by mucoadhesion. Chemical modifications of drugs to enhance absorption are not described in this chapter.

In certain instances, for example, with the use of the gum from *Hakea gibbosa*, it may not be totally clear to what extent enhancement of absorption is a resultant of traditional permeation enhancement, simply due to mucoadhesion, or to a reduction in drug metabolism. Chitosan, which has traditionally been considered a permeation enhancer, is thought to also have multiple effects. Since it can be difficult to tease out the extent of each effect in some instances, it makes sense to consider all three effects in a description of methods to improve delivery of drugs by chemical means. This approach has been followed in this chapter which considers all three types of action: traditional permeation enhancement, mucoadhesion, and suppression of enzymatic breakdown.

3.2 The Ideal Absorption Enhancer

Although different types of enhancers have been mentioned, the following ideal characteristics are desirable for all of them:

a. The enhancer should provide the desired degree of enhancement, usually at least 20 % and frequently several 100 %.
b. The amount of absorption enhancer to be used is relatively small so that a dosage form of reasonable size may be formulated.
c. Ideally, the absorption enhancer has no side effects, or side effects are so limited that the material is well tolerated by the patient.
d. Recovery from any tissue damage is rapid.
e. Side effects do not interfere in a significant way with the patient's life style. If the taste of food were affected, this would not, generally, be considered a serious side effect. Yet, the impact of a regularly taken drug that affects taste may be considered to be significant by a patient for whom the enjoyment of food is important.
f. The enhancer should not, itself, be absorbed. If the absorption enhancer is absorbed, then attention should be focused on its effects on the entire body and not just at the site of absorption. A mild response at the site of absorption, which disappears in a few hours, is far easier to defend with regulatory authorities than a more serious systemic effect.
g. Repeated administrations of the enhancer (as a part of a drug/enhancer combination) should be acceptable. Many drugs require repeat administrations and if the enhancer can be applied to the same area fairly frequently, its utility would be greater. For example, if the recovery time of the tissues, after drug application to the buccal mucosa, is 16 h, a once-a-day medication can be applied to alternate cheeks, with recovery before the next application.
h. The absorption enhancer should have no taste. Drug delivery via the oral cavity may, typically, be a low-dose drug, e.g., 1 mg. Thus, the permeation enhancer may well be present in a much higher amount and, possibly, have a dominant effect on the taste of the dosage form. In view of the fact that the taste of the dosage form should be acceptable for this route of delivery, it becomes imperative for the enhancer not to contribute a significant taste. It is clear that the taste should not be bad (in order to ensure compliance) but the taste should also not be excessively pleasing since this would promote salivation, potentially increased drug release, and swallowing of the dissolved drug with the saliva.
i. The absorption enhancer should not leave a residue that is perceived as unpleasant, for example, leave a chalky feel. This may be important in the case of mucoadhesives, a portion of which may remain after the drug is released. The residue should either dissolve (and be swallowed) or should be easily removed, by some other means, from the application site.

3.3 Traditional Permeation Enhancers

A permeation enhancer is a chemical compound which is added into the formulation along with the target drug in order to improve permeation through the biological membrane. The enhancer should not cause unacceptable damage or toxicity to the membrane or underlying tissues. Numerous studies have been conducted to find permeation enhancers for drug delivery through the skin, nasal, and intestinal mucosae. In recent years, many of these compounds were also investigated for a similar effect on drug absorption through the oral cavity mucosa [2]. In the following sections, some of these compounds will be discussed in more detail with specific emphasis on the magnitude of their enhancing effect and the proposed mechanisms of action.

3.3.1 Bile Salts

Bile acids are produced in the liver and they are later converted into salts which have a natural function to promote absorption, in the small intestine, of lipids from the diet. In in vitro studies, bile salts have been shown to enhance absorption of drugs through the nasal, rectal, pulmonary, and vaginal epithelia [3]. Bile salts are thought to enhance buccal permeation by extraction of membrane proteins and lipids, membrane fluidization, and intercellular lipid extraction. Many researchers have conducted studies to show the effects of bile salts and many have achieved good improvements in drug permeation. For example, Şenel and coworkers studied the enhancing effects of dihydroxy and trihydroxy bile salts on the buccal permeation of fluorescein isothiocyanate (FITC). At 0.1 M concentration, the permeability of FITC increased 100–200 times. Using a light microscope and freeze-fracture electron microscopy, significant morphological and ultrastructural changes were observed after treatment with bile salts. The dihydroxy and trihydroxy bile salts were not significantly different with respect to both the absorption enhancement ratio and the morphological changes [4]. In a similar study, Hoogstraate and coworkers used bile salts to improve the absorption of FITC and FITC-labeled dextrans through porcine buccal mucosa [5]. Both dihydroxy and trihydroxy salts were used. Sodium glycodeoxycholate (GDC) and sodium taurodeoxycholate (TDC) fall into the former group, whereas sodium glycocholate (GC) and sodium taurocholate (TC) are examples of the latter.

At 100 mM concentration of bile salts, FITC absorption was enhanced by a factor of 200. Also, a marked increase in the depth of permeation of FITC-dextrans through biological tissue was shown. Larger molecular weight (MW) dextrans, however, required higher concentrations of bile salts to enable permeation of the dextran. Microscopic studies revealed that at low concentrations (2, 5, and 10 mM), bile salts increased fluidity of lipids in the intercellular compartment. Interestingly, when the concentration of enhancer was raised to 100 mM, it affected the lipids in both the intercellular and intracellular compartments. While it may appear

appealing to use a higher concentration and, thus, involve multiple mechanisms to enhance absorption, in practice, higher concentrations also cause more side effects. A lag time in the absorption of FITC-labeled dextrans was observed. Also, the tissue concentrations of FITC and FITC-dextrans did not increase linearly as the concentrations of bile salts were increased, but rather the absorption curves were sigmoidal. This suggests that a particular critical concentration of bile salts in the tissue needs to be reached before an enhancing effect can occur. Then, further increases in concentration of bile salts enhance absorption up to a point, beyond which further enhancing effects are not significant.

Working with bovine buccal mucosa, Şenel and coworkers demonstrated the enhancing effect of sodium GDC on morphine sulfate [6]. An enhancement factor of 5 was demonstrated at 100 mM concentration of GDC, whereas no enhancement was seen with 10 mM GDC. In a similar study, the effects of sodium glycocholate (GC) on the permeation of morphine hydrochloride (MPH) were observed [7]. A major difference in this study's design was the use of porcine buccal mucosa in place of bovine. At 100 mM concentration, GC enhanced the permeation of MPH by a factor of 2, while at 10 mM concentration, no effect on permeation was observed. Furthermore, a lag time of 1 h was observed between the permeation of GC into the tissue and the permeation enhancing effect of GC towards the permeant. The authors state that it seems that a certain level of GC accumulation in the tissue is necessary for enhancement to occur. It is also possible that the enhancer accumulates faster than the indicated 1 h, but that it takes some time for the enhancer to bring about the desired changes, such as extraction of membrane components or extraction of intercellular lipids.

While bile salts show great potential in enhancing drug permeation through the buccal mucosa, their safety has been questioned. Since these compounds are inherently irritating, their impact on mucosal tissues is an important concern when they are used as buccal permeation enhancers. Since most drugs require one, or more, administrations per day, the buccal tissue will not have much time to recover after each use.

In a study carried out to determine the safety of bile salts as permeation enhancers [8], bioadhesive tablets were prepared with 5% GDC and tested on healthy volunteers. The bioadhesive tablets were applied to the buccal mucosa and left in place for 4 h to determine the extent of mucosal irritation. It was found that the epithelial cells required at least 24 h to recover. Since many drugs are administered 12- or 24-hourly, GDC may not be suitable as a permeation enhancer. Additionally, frequent damage to rapidly dividing tissues, such as the buccal mucosa, could lead to point mutations, which may result in the formation of cancerous cells.

In addition to permeation enhancement, a reduction in the proteolytic rate of peptide substrates was seen in tissue homogenates in the presence of sodium GC [9]. At maximum effect, a fivefold reduction in proteolysis was observed in the presence of bile salts. This inhibitory effect was considered to further contribute to the effective amount of drug that permeates the mucosa. However, there are additional considerations which are discussed in Sect. 3.7 which deals specifically with the topic of enzyme inhibition.

3.3.2 Fatty Acids

While a number of studies have demonstrated the enhancing effect of fatty acids on drug permeation through the buccal mucosa, the exact mechanisms by which fatty acids elicit this effect have not been fully elucidated. Fatty acids are thought to interact with the phospholipids of cell membranes. The fatty acid monomers become inserted between alkyl chains of cell membrane phospholipids and, due to an imperfect fit, enhanced fluidity of the membrane results. This allows greater drug permeation, resulting, ultimately, in increased drug diffusion via the transcellular route.

Ganem-Quintanar and coworkers [10] described the ideal characteristics of fatty acids to promote maximal enhancement of mucosal permeation. The length of the fatty acid chain appears to have a notable influence: fatty acids containing between 6 and 14 carbons are effective. When these "medium-chain" fatty acids are unsaturated, they are usually more disruptive of the permeation barrier (cell membrane) than an equivalent saturated fatty acid [11]. The configuration (*cis* or *trans*) and the site of the unsaturation can be significant. Cooper [12] found that fatty acids with the *cis* configuration are more effective enhancers compared to their *trans* counterparts. The fact that the *cis* isomer has a "kink" in its structure, and is less linear than the *trans* isomer, is probably the reason for its greater effectiveness in disrupting the arrangement of membrane lipids. Thus, drug permeation is enhanced to a greater extent.

Double bonds, with *cis* configuration, that are located close to the middle of the carbon chain have a more disruptive effect. This is due to the difficulty experienced by cell membrane alkyl chains to pack closely around the fatty acid [10]. In addition, more elaborate branching of fatty acids, i.e., the presence of multiple double bonds, can possibly increase the enhancing effect. However, very large lipids with multiple branches might have difficulty penetrating into, and being accommodated within, the cell membrane, thus losing their potential to enhance permeation. Ionization of a fatty acid can have a negative effect on its permeation enhancing ability, i.e., undissociated fatty acids can fluidize lipid bilayers better than the same fatty acid in the ionized state [10]. This fact substantiates the theory that fatty acid molecules insert themselves between the alkyl chains of the cell membrane phospholipids, and not between the polar heads.

The effects of cod-liver oil extract (CLOE) and oleic acid, on the permeation of ergotamine through the mucosa of the hamster cheek pouch, were investigated [13]. There was no significant difference between the enhancing effects of CLOE and oleic acid. This was expected since CLOE is a collection of fatty acids including oleic acid. The partition coefficient values of the two substances were not statistically different. A substantial increase in the flux of ergotamine was seen when either enhancer was applied to buccal mucosa. The permeation of ergotamine was highest with the addition of 5% cod-liver oil or 3% oleic acid. When the concentration of enhancers was further increased, the flux decreased, indicating a complex interaction between the enhancer, the cell membrane, and the drug. These permeation enhancers increased the permeation of the unionized form of ergotamine more than

that of the ionized form. Since the hamster cheek pouch has a keratinized mucosa, inferences about the permeation enhancing effect of CLOE and oleic acid on human buccal tissues cannot directly be drawn.

In an interesting study, the permeation enhancer, oleic acid, was used in combination with polyethylene glycol 200 (PEG 200). The studied drug was [D-Ala, D-Leu] encephalin (DADLE) and attempts to enhance its permeation through porcine buccal mucosa were investigated [14]. Glyceryl monooleate in the cubic phase served as the vehicle. In a previous study by the same authors, oleic acid was used as the enhancer without the addition of PEG 200. The results showed that very little oleic acid was able to leave the cubic phase vehicle to enter the buccal tissue. The addition of PEG 200 to the formulation increased the aqueous solubility of oleic acid which was able to leave the vehicle more readily. This resulted in much more oleic acid entering the tissue and this enhanced the permeation of DADLE. The PEG 200 was shown to be located in the cubic phase, a requirement for its solubilizing effect on oleic acid. A significant increase in DADLE permeation into the tissue was noted and the authors conclude that PEG 200 enhanced the action of the permeation enhancer (oleic acid) and, therefore, that this combination can be a useful tool to improve buccal membrane permeability of peptide drugs.

The value of using two substances to promote permeation (enhancer and coenhancer) was demonstrated in another study where propylene glycol served as the coenhancer of oleic acid, used to improve propranolol permeation through excised porcine buccal membrane [15]. With propylene glycol and oleic acid concentrations in the range of 1–10%, there was a three- to fourfold increase in the permeability of propranolol. Propylene glycol also appeared to decrease the lag time between the administration of oleic acid and the enhancement of permeation. The observed lag time is considered to be a disadvantage of the use of fatty acids to improve drug absorption. Since propylene glycol was able to decrease this time, it is considered a desirable additive or coenhancer. Light microscopic studies revealed no apparent tissue damage from the propylene glycol and oleic acid applications.

3.3.3 Chitosan

Chitosan has come into prominence in the past 20 years because of its ability to enhance drug delivery through various tissues. Its ability to promote absorption through the intestines and nasal mucosa has been demonstrated [2]. This biocompatible and biodegradable polymer is made by treating crustacean shells with sodium hydroxide. Chitosan is described as a linear polysaccharide composed of randomly distributed β-(1–4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan is postulated to enhance absorption by several mechanisms. It is possible that multiple mechanisms are at play in a single situation, each contributing to the total effect, as also seen with the natural polysaccharide in the exudate from *H. gibbosa*. It is also possible that one mechanism may predominate in some applications. Some authors attribute the enhancing

effect to the mucoadhesive nature of chitosan which results in increased retention of the dosage form to the buccal mucosal surface, simply providing a longer period for drug permeation, as discussed in greater detail in Sect. 3.6.

Noting that chitosan was able to open tight junctions in the intestinal epithelium through charge-dependent binding (thereby allowing better drug absorption), researchers have postulated a similar mechanism in the oral cavity. Since human buccal mucosa does not have many tight junctions, this theory was questioned. However, gap junctions and desmosome junctions predominate in the oral mucosa, and tight junctions and desmosome junctions are partly regulated by calcium ions. An increase in calcium ion concentration signals junction closure, and a dearth of calcium ions signals junction opening. If chitosan's ability to open tight junctions in the intestine is due to its calcium-binding effect, it is reasonable to postulate that a similar effect could occur at desmosome junctions in the oral cavity. It has also been suggested that the enhancing effect of chitosan could be due to its disruptive effect on intercellular lipid packing in the buccal epithelium [2].

As mentioned, chitosan has received increasing attention as an oral mucosal permeation enhancer over many years. As may be expected, earlier studies focused on basic effects, such as mucoadhesion and the ability to enhance permeation of small molecules; later, its utility for enhancing the absorption of peptides and other macromolecules was determined. Since basic chitosan applications and properties have been well described in the previous literature and in review articles, only a few such studies will be mentioned. Then, selected studies revealing newer applications will be described.

Examples of earlier studies include an illustration of the directly compressible nature of chitosan [16], and mucoadhesion, rapid drug release rates from the dosage forms, and good bioavailability [17]. The latter study used a combination of chitosan and sodium alginate with diltiazem as the drug. An early example of the use of chitosan as an enhancer for peptide absorption is the study of the permeation enhancement of transforming growth factor-β (TGF-β), a large bioactive peptide, across porcine buccal mucosa [18]. While the oral mucosa is reported to be relatively impermeable to TGF-β, results of this study showed a six- to sevenfold permeability enhancement by chitosan. It was postulated that the hydrophilicity of the compound was important for absorption enhancement by chitosan, since the absorption of hydrocortisone, a hydrophobic, water-insoluble compound that had previously been tested, was not enhanced by the addition of chitosan. After exposure to chitosan gel for up to 8 h, microscopic examination of histological sections of porcine buccal mucosa did not show any damaging effects [19].

Examples of more recent, novel applications of chitosan for oral mucosal drug delivery are now described. Insulin-loaded nanoparticles consisting of poly(ethylene glycol)methyl ether-block-polylactide (PEG-b-PLA) tend to wash away with the saliva when applied to the oral mucosa, thus limiting the utility of an otherwise good mechanism to deliver this peptide. Therefore, Giovino et al. [20] embedded these nanoparticles in a chitosan thin film. The latter will adhere to the mucosa, reducing the tendency for particles to be washed away. Ayensu et al. [21] produced porous chitosan-lyophilized wafers as a mucoadhesive drug delivery system

for protein drugs, using bovine serum albumin (BSA) as the model drug. They subjected the wafers to dialysis in order to remove sodium acetate, formed when sodium hydroxide is added to neutralize acetic acid (the solvent for chitosan). In the absence of dialysis, the crystallinity of the wafer was high which had the effect of increasing brittleness, decreasing mucoadhesion and, also, decreasing the rate of BSA release [22].

Pongjanyakul et al. [23] used film casting and solvent evaporation to prepare chitosan–magnesium aluminum stearate (MAS) films containing nicotine. Due to the electrostatic complexation of MAS with nicotine as well as with chitosan, nanocomposites were formed within the film. This structure prevented the rapid volatilization of nicotine during heating to dry the film, and it also provided a slower release of the drug, in an acidic medium, compared to a film with chitosan alone. MAS is responsible for cross-linking chitosan and this could have contributed to the slower drug release. The nanocomposite structure was confirmed using X-ray diffraction. Of importance is the fact that the inclusion of MAS did not reduce the mucoadhesion of the polymer.

Abruzzo et al. [24] produced films consisting of chitosan mixed with gelatin in different proportions (not a laminated film). The motivation for doing so appears to be the fact that chitosan, while displaying good mucoadhesion, absorbs water very slowly and, thus, releases the drug slowly. This was unsuitable for the authors' intended use of administering propranolol buccally for the treatment of hypertension and atrial fibrillation. Gelatin, on the other hand, absorbs moisture rapidly with the result that it completely dissolves in 10 min. By using appropriate mixtures of the two polymers, the required release rate could be obtained. With the incorporation of mannitol, complete drug release, and a suitable in vitro drug permeation rate through porcine buccal mucosa, could be assured. Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) studies confirmed the interaction between chitosan and gelatin in the films, probably by the formation of ionic bonds. DSC and scanning electron microscopy (SEM) also revealed the absence of drug crystals. While films consisting exclusively of chitosan did not show uniformity of drug content, weight, or thickness, the combination films did. Volunteers did not experience irritation during or after the study and the film did not inhibit probiotic organisms.

Sandri et al. (2005) examined the effect of N-trimethylation of chitosan on its mucoadhesive properties and permeability enhancement. They found that higher degrees of N-trimethylation increased the mucoadhesion and permeation enhancement in pH 6.4 phosphate buffer but not in water. The mucoadhesion increased in spite of a lower solution viscosity. The test permeant was FITC dextran, a hydrophilic molecule with an MW of 4400 Da which is known to permeate via the paracellular pathway. Since peptides, in general, also permeate via this pathway, the authors feel that this chitosan derivative is of importance in the delivery of peptides. The authors postulated that the polymer permeation into mucin reduces the absorption barrier, perhaps disrupting the extracellular matrix as well as weakening the intercellular bonds. The authors state that the mucoadhesive effect, thus, cannot

be separated from the permeation enhancing effect, a view shared by the present authors.

The fact that chitosan is a positively charged polymer which leads to many of its desirable features is stressed in a recent review article [25]. The article also refers to the fact that the cationic groupings provide the option of chemical modification, with further potential enhancement, and summarizes the advantages of using chitosan in various delivery systems.

3.3.4 Surface Active Agents

Surface active molecules, or surfactants, have balanced polar and nonpolar groups. They usually contain a small polar head, which is attracted more to the hydrophilic phase, and a long nonpolar tail, which is attracted more to the hydrophobic phase, of a mixture. This structure endows them with special properties at surfaces and at interfaces. At the interface between two immiscible liquids, they reduce the interfacial tension enabling, for example, the preparation of emulsions. In contact with biological systems, the lipophilic portions of surfactant molecules may interact closely with the lipids of cell membranes. An appropriately selected surfactant may enter into the structure of the lipid membrane. This disrupts the regular packing of the lipids forming the cell membrane and, thereby, compromises its integrity. This allows applied drugs to permeate through those areas of the cell membrane that have been disrupted in this way. Surfactants may also extract proteins from cell membranes, compromising the integrity of the membrane in this way as well.

The enhancement of permeation due to the disruption of the integrity of the membrane is, obviously, not restricted to the drug of choice. Other substances, including toxic substances, may permeate more freely. Since membranes allow for the selective flow of ions into, or out of, the cell, any change in the transmembrane ion gradients my cause ill effects. Ideally, the membrane would be disrupted minimally yet allowing adequate permeation of the applied drug. Then, the membrane would be spontaneously repaired at a sufficiently quick rate such that the untoward effects of membrane disruption are minimal.

Sodium lauryl sulfate (SLS), which is also known as sodium dodecyl sulfate, is probably the surfactant that has been tested the most for its permeation-enhancing effects. Often, only a low level of enhancement was achieved and some toxicity was also observed. Working with Idebenone, a synthetic analogue of coenzyme Q10, it was found that the addition of 4% SLS enhanced permeation through bovine buccal mucosa in vitro to the extent of only 1.54 times, whereas hydroxypropyl cyclodextrin increased the permeation rate by 45 times [26]. On the other hand, another study showed that the absorption of salicylic acid through keratinized buccal mucosa (hamster pouch) increased with the pretreatment of the tissue with SLS or cetylpyridinium chloride at different pH levels between 3 and 7, and that enhancement was dependent on the concentration of the surfactant used [27]. In this study, decreased absorption was observed with the use of polysorbate 80 at lower

pH conditions. This was attributed to the interaction of the surfactant with the drug and, consequently, a lower concentration of free drug.

The ability of several alkylglycosides to enhance permeation through different mucosal tissues was studied, using the pharmacologic effect of insulin (lowering of glucose level) as an indicator of the absorption of this model, poorly available protein [28]. Octylglucoside and dodecylmaltoside (5 %) had a greater enhancing effect on buccal, than on nasal or rectal, absorption. At a level of 1 %, the latter enhancer increased insulin absorption slightly.

Rai and coworkers studied Tween 80 and Brij 58, both nonionic surfactants, for their ability to promote the permeation of naltrexone through engineered human buccal tissue [29]. They found that Tween 80 reduced permeation to a slight extent whereas Brij 58 increased permeability approximately sixfold. Upon histological examination, no tissue damage was observed.

3.4 Inclusion Complexes, Dendrimers, and Micelles

These three enhancers are considered together as they all may hold a drug molecule within their structure. If they are able to carry the drug across a biological membrane and then release it, they would have enhanced the drug permeation of that membrane.

Some large organic molecules have a space within the structural core in which a smaller molecule may fit to form an inclusion complex. On the other hand, a drug may have physicochemical properties that dictate that it will not cross a biological membrane to a significant extent. Within the host molecule, such a drug or "guest molecule" adopts the properties of the carrier and may, thus, be able to cross biological membranes. Cyclodextrins are possibly the best studied of the "host" molecules capable of forming such inclusion complexes.

Hydroxypropyl-β-cyclodextrin (HPβCD) can serve as a host to a poorly soluble molecule since the cavity is hydrophobic and can accommodate hydrophobic guest molecules. The complex will cross biological membranes more easily than the drug (guest) alone. Rathi and coworkers demonstrated that a drug–cyclodextrin complex can increase the apparent aqueous solubility, dissolution rate, and permeation rate of a drug through bovine buccal tissue [26]. Idebenone, a synthetic analogue of coenzyme Q_{10}, is poorly soluble and has a low bioavailability through the oral route. Idebenone complexed with HPβCD showed a good flux and permeation enhancement. When compared with different permeation enhancers, HPβCD was the most potent, increasing permeation by an enhancement ratio of 45. This led to the conclusion that the inclusion complex can, itself, act as a permeation enhancer for buccal drug delivery of idebenone and that there was no need for additional enhancement mechanisms. Improving the apparent water solubility of poorly water-soluble drugs by complexation with cyclodextrins and then delivering these via the buccal or sublingual mucosa may be advantageous for increasing drug absorption, especially with hydrophobic drugs.

Dendrimers are large molecular entities comprised of branching chains made from a monomer and linker. The entity can become quite large and has numerous open spaces, the so-called dendritic box, in which drug molecules can be trapped. Polyamidoamine (PAMAM) dendrimers are the most popular and have been demonstrated to cross the intestinal barrier [30]. Consequently, they have been proposed as drug delivery systems, as exemplified by a study of the permeability of ^{125}I-labeled dendrimers through everted rat intestinal sacs in vitro [31]. The time course of the appearance of radioactivity in the tissue and serosal fluid was followed. Following successful demonstrations of permeation enhancement, dendrimers have, more recently, been suggested as permeation enhancers for buccal delivery.

With the aid of Frantz diffusion cells, Yuan et al. [32] illustrated that several types of dendrimers were able to permeate porcine buccal mucosa. The well-characterized opioid peptide drug, D-penicillamine2-D-penicillamine5-enkephalin (DPDPE) and, in certain examples, FITC (or other labels) and a targeting ligand were bonded to preformed (commercial) dendrimers. While the dendrimer enhanced drug uptake, coadministration of a mucoadhesive and a bile salt further enhanced absorption of the opioid drug. Although brain uptake studies were not conducted, the authors concluded that buccal delivery showed promise as an alternate mechanism, to IV injection, to deliver central nervous system (CNS) drugs.

When a surfactant is added to a suitable solvent, e.g., water, in sufficient concentration, some of the molecules arrange themselves into aggregates with the polar heads facing the exterior medium (water) and the nonpolar tails facing inward. The concentration at which this occurs is referred to as the critical micelle concentration (CMC). Below the CMC, the surfactant molecules remain dispersed as individual molecules. By formation of the aggregates, the hydrophobic tails resist contact with water and, in so doing, create a hydrophobic environment within the core of the micelle. This environment is suitable for the incorporation of hydrophobic drugs. Micelles may also be formed from more than one surfactant, in which case they are referred to as mixed micelles. Depending on the surfactants used, hydrophobic or hydrophilic drugs may be trapped within the micelle. The Generex company uses mixed micelles to deliver drugs, especially macromolecules, through the buccal mucosa. The mixed micelles are formed from an alkali metal alkyl sulfate, and at least three different micelle-forming compounds, as described in US patents [33–35]. The micelle size is less than about 10 nm. Insulin is one example of a macromolecule that is mentioned in the patents as being suitable for administration in this manner. A proof of concept study demonstrated that a buccal spray, formulated in accordance with these principles, reduced post prandial hyperglycemia in obese subjects with impaired glucose tolerance [66].

3.5 pH Control and Effervescence

In a series of papers from Beckett's laboratory, the workers demonstrated that some drugs could be absorbed from the oral cavity (this fact was not taken for granted in the 1960s). Further, the importance of pH control on the absorption of drugs from

the oral cavity was demonstrated (for example, [36, 37]). Despite the elegance of the experiments (by prevailing standards) and the import of the results, this observation was not translated into transmucosal products.

The use of effervescence has been suggested as a promoter of oral transmucosal permeation stemming from in vitro experiments involving excised tissue applied between Ussing chambers. The method involved pumping vast quantities of CO_2 through the donor chamber [38]. This experimental method achieved modest permeation enhancement and the postulated mechanisms for enhancement were: solvent drag effect, opening of tight junctions, changing the pH of the microenvironment, and increasing the hydrophobicity of the tissues due to permeation by CO_2.

Pather et al. [39] studied the effects of pH control and effervescence when applied to poorly soluble weak bases. With fentanyl, a therapeutically significant enhancement of absorption (relative to an unenhanced formulation) was observed with buccal placement of the dosage form. The blood levels following sublingual placement of the tableted dosage form were very similar.

The combination of water and CO_2 (from the effervescence reaction) produced carbonic acid. It was postulated that the resulting lower pH promoted the formation of the ionized species of the drug and, since the ionized species has a greater solubility, more rapid dissolution of the drug occurred. Over several minutes, the solution's pH then becomes less acidic as CO_2 is released from solution into the atmosphere. Alternately, the CO_2 may be absorbed into tissues. As the pH increases, more of the dissolved drug is converted to the nonionic form, which is more permeable. This pH transition allows the drug to rapidly dissolve initially (ionized form) and then to slowly convert to the nonionized form for good permeation into biological tissues. The range of pH transition can be modified by the addition of additional pH modifiers. This represents ideal conditions for drug transformation from a crystalline salt within a tablet to the ionized, soluble form in solution to, finally, the unionized, permeable form in solution. The latter enables rapid absorption into tissues. In human studies, this effect led to faster drug absorption than similar tablets without the absorption enhancers (pH modifiers and effervescence; [39]). Much larger studies confirmed the observation that buccal, or sublingual, placement of the tablet resulted in similar blood levels of the drug [65].

Singh and Pather [40] developed sublingual tablets containing zolpidem and a buffer system. The disintegration rate of the tablet was designed to be relatively rapid and controlled in order to release the drug in a predetermined manner. The drug formulation is intended for the treatment of middle-of-the-night insomnia, a new indication. The buffer enhances the absorption of the drug. Due to the possibility of rapid absorption, it is mandatory that the product be taken with the patient in bed since there is the possibility of rapid onset of the hypnotic effect. As a result of the rapid absorption by the sublingual route, the patient falls asleep rapidly; due to the low dose and rapid clearance, the patient awakes after approximately 4 h with no residual effects. In a next-day driving study, subjects fared very well with respect to side-to-side movements within a lane when monitored by a computerized system [41]. The drug has a longer half-life in women, who must take half the dose. As a result of these findings, the Food and Drug Administration (FDA) has mandated

that all zolpidem products should have new labeling, reflecting the different clearance rates between men and women [42].

In a study on a series of beta-blockers of varying lipophilicity, Wang et al. [43] studied the permeability of the drugs through cell culture and freshly excised porcine buccal mucosa when the following parameters were altered: osmolarity, sodium GDC addition, and pH adjustment to a value higher than the pK_a of the respective drug. The effect of the three modifiers was greatest on the most hydrophilic of the series of drugs (atenolol), and least on propranolol, the most hydrophobic of the series. Of the three enhancers, pH had the greatest effect and was considered suitable as an enhancing mechanism in drug delivery.

Wang et al. [44] utilized the concept of pH_{max}, i.e., the pH at which the sum of the ionized species and the unionized species in solution is at a maximum. For a drug such as propranolol, which is capable of transcellular as well as paracellular transport, the use of the pH_{max} concept proved valuable in that a tablet buffered to pH_{max} achieved a higher area under the curve (AUC) in the first 30 min than a nonbuffered tablet when both were administered sublingually to human subjects.

3.6 Mucoadhesives

For oral cavity delivery, mucoadhesives have historically been used to retain the dosage form to the mucosa, thus enabling a longer period for the drug to permeate the tissues. A drug has to have some potential to be absorbed for the mucoadhesive to improve drug delivery. If the drug has extremely low permeability, holding it adjacent to the mucosa for an extended time and affording it the opportunity to be absorbed may not actually enhance absorption to a therapeutically meaningful extent. Traditionally, the mucoadhesive has been incorporated into a tablet or disc which is pressed onto the mucosal surface, such as the buccal mucosa, often after slight moistening of the tablet. The added moisture, or saliva, hydrates the mucoadhesive polymer incorporated into the dosage form, causing it to adhere to the mucosal surface. Optionally, the tablet may be coated with an impermeable material on all-but-one flat face of the tablet [45]. This technique was employed by Alur and coworkers to develop mucoadhesive tablets of calcitonin [46] and chlorpheniramine maleate [47] with good mucoadhesion, sustained release, and extended plasma half-life in rabbits. There are many examples of a similar use of mucoadhesive polymers, including chitosan which is, possibly, the most extensively described mucoadhesive. This topic has been extensively reviewed previously, for example, the article by Shaikh and coworkers which provides a general description of mucoadhesion, discusses the theories by which this phenomenon occurs, and provides a description of mucoadhesion to various mucosal tissues in the body [48]; and that by Sudhakar and coworkers which is more specific to the oral mucosa [49]. This section will, therefore, focus on more recent, novel applications of the basic mucoadhesive concept for oral mucosal delivery beyond the traditional dosage forms.

Sander et al. [50] described the development of chitosan-based bioadhesive microparticles containing the antidiabetic drug, metformin hydrochloride. A series of microparticles were prepared by spray drying aqueous dispersions containing chitosan and metformin in different ratios, and by utilizing chitosan grades of increasing MW. They observed an increase in retention of metformin-containing microparticles on porcine mucosa with increasing chitosan: metformin ratios. On the other hand, higher chitosan MW fractions did not have a similar effect on the resultant microparticles. The authors conclude that metformin-containing chitosan microparticles with significant retention to porcine buccal mucosa were successfully prepared. Determination of the absorption of metformin through the mucosa will be the subject of a later study by these researchers. The fact that metformin is water soluble means that it will easily elute from a conventional dosage form and be washed into the stomach where it has the potential to cause irritation. The mucoadhesive microparticles have a lesser tendency to leak the drug. Presumably, the microparticles cover a larger surface area of the mucosa than is practical with tablets or patches.

3.7 Enzyme Inhibitors

Since peptides may be metabolized to some degree in the oral cavity, the coadministration of an enzyme inhibitor could slow down the metabolism of the drug sufficiently to allow absorption to a reasonable extent. Aungst [51] has illustrated that different enzymes can attack a peptide at different positions (cleave different bonds) and that specific inhibitors can block each of these reactions. Exopeptidases attack the ends of the peptide (with carboxypeptidases affecting the carboxylic end and aminopeptidases, the amino-containing end). Endopeptidases lyse the peptide along the length of the peptide and not its ends. Boroleucine, Amastatin, ethylenediaminetetraacetic acid (EDTA), and puromycin are examples of enzyme inhibitors that have been studied in relation to the suppression of metabolic enzymes. In addition, Hao and Heng [52] made reference to Aprotinin and Bestatin which also have specific enzyme-inhibiting effects.

The classical method to study whether a specific tissue is capable of enzymatic breakdown of peptides is to homogenize the tissue and incubate it with the peptides of interest. If the concentration of the starting peptides is reduced, metabolism is said to have occurred. Likewise, if a suspected inhibitor is added to the drug and homogenate, and decreased metabolism of the peptide ensues, it is said to reflect the effectiveness of the inhibitor. This is the method used in the pioneering work of Lee [53, 54]. In relatively newer work, Alur et al. [55] used tissue homogenates to demonstrate the metabolism-inhibiting effect of the gum from *H. gibbosa* in homogenates of rabbit buccal mucosa.

Yamamoto et al.[9] studied the proteolytic activity of various absorptive mucosae, as well as the inhibitory effect of several enzyme inhibitors using homogenates of rabbit tissues. Interestingly, the proteolytic activity was highest in the nasal and

rectal homogenates, and lowest in the buccal homogenates. The rate of insulin and proinsulin proteolysis in the buccal mucosa was about one sixth that of the nasal. A concentration of 0.01 % aprotinin reduced the metabolism of insulin and proinsulin in buccal mucosa homogenates by 70–80 %.

The problem with the approach of using homogenates is the fact that the peptidase in question may be contained intracellularly (within the cytosol) whereas peptides are generally thought to permeate the tissue via the paracellular route. An enzyme contained within the cell cannot metabolize a drug outside of the cell (within the intercellular space, for example). This is a phenomenon that may be described as the "lion-in-the-cage effect." A lion is able to destroy humans, but people may walk freely (permeate) throughout the zoo, so long as the lion remains in its cage. What is the evidence that metabolizing enzymes are mainly contained within cells?

Aungst and Rogers [56] studied the effects of a number of enhancers on the absorption of insulin by observing the hypoglycemic effect induced in rats during the first 4 h after administration. The efficacy of the enhancer was rated by comparison of the observed hypoglycemic effect to that of intramuscular insulin. Unenhanced insulin administration by the buccal route resulted in low absorption, as judged by the relative magnitude of the induced hypoglycemic effect. Laureth-9 improved insulin absorption upon buccal administration; aprotinin, an enzyme inhibitor, did not. In addition, when aprotinin was coadministered with laureth-9, no further increase in drug absorption was seen. Since aprotinin did not enhance absorption of insulin, it could not have suppressed enzymatic degradation.

Aprotinin's efficacy as an enzyme inhibitor has been demonstrated in numerous studies utilizing buccal homogenates, for example, by Lee [53, 54]. In the Aungst and Rogers study, laureth-9 enhanced insulin absorption to about 30 % of the intramuscular level. Hence, sufficient insulin was passing through the mucosa to provide a substrate for enzyme degradation, if metabolizing enzymes were, in fact, present. It therefore appears likely that proteolytic enzymes are not present in the intercellular space and that damage to the cell structure, with consequent spilling out of enzymes from within the cells, is required for enzymatic degradation. Hence, inhibition of enzyme activity, with consequently enhanced permeation of insulin, could not be demonstrated in this model. This work supports the idea that these enzymes are contained within the cells. The authors point out that there may, additionally, be enzymes which metabolize the drug in the blood and at the site of action. These, however, affect the duration of action of the drug and not its absorption which is the subject of this discussion.

Since bioadhesive systems bring high concentrations of the drug into close contact with the buccal mucosa, Walker et al. [57] examined the enzyme activity of the surface of pig buccal mucosa. No endopeptidase, carboxypeptidase, or dipeptidyl peptidase IV activity was observed. However, aminopeptidase N activity was detected when Leu-*p*-nitroanilide served as the substrate. Insulin and the insulin B chain were stable for 2 h when in contact with the mucosa. Leu-enkephalin underwent substantial metabolism which was reduced in the presence of the aminopeptidase inhibitors, amastatin, sodium deoxycholoate, and EDTA. The authors conclude

that the metabolism of administered drugs could best be estimated by a study of enzyme activity on the surface of the mucosa, not by homogenization.

Dowty et al. [58] and Johnston et al. [59] used excised mucosal tissue in diffusion cells and studied the level of degradation of the drug on both the mucosal and the serosal sides of the membrane (donor and receiver compartments, respectively). Endopeptidase activity was observed on both surfaces. The enzyme activity on the mucosal surface was said to be due to membrane-bound enzyme, whereas no explanation for the serosal effect was offered. It seems probable that the endopeptidase activity on the serosal side was due to damage to the underlying cells during surgical excision with consequent release of enzymes.

The mechanisms of inhibition by peptidase inhibitors are not fully understood and may include multiple mechanisms such as altering the conformation of the drug, and making the drug sterically less accessible to the metabolizing enzyme, apart from directly affecting the activity of the enzyme. The fact that some bile salts have peptidase-inhibiting effects illustrates the difficulties with categorization of absorption enhancers since bile salts have long been considered to act by membrane fluidization, intercellular lipid extraction, and by extracting proteins and lipids from cell membranes. Attenuation of the metabolizing effects of enzymes may be an additional mechanism of action of bile salts.

In addition to an absorption enhancer (sodium glycocholate), Johnston et al. [59] utilized guanidine hydrochloride as an agent to unfold the peptide substrate, basic fibroblast growth factor. They found that the order of addition of these substances to the donor diffusion cell (with a lag time between additions) was important. If guanidine hydrochloride was added first, there was a significant enhancement of absorption but not if it was added after the addition of sodium GC. In this work, it is presumed that the more linear conformation, rather than the globular, results in enhanced permeation of the peptide. When added first, guanidine hydrochloride produced the linear conformation of the peptide, and its permeation was enhanced by the subsequent addition of sodium GC.

Chitosan complexed with EDTA to form chitosan–EDTA is a potent inhibitor of metallopeptidases such as amino peptidase N in porcine intestinal mucosa [60]. Zinc is an essential cofactor for aminopeptidase N. Chitosan–EDTA was able to bind zinc (binding capacity approximately 2 mmoles of Zn per gram at pH 6.5) and the activity of 48 mU/mL of the enzyme was completely inhibited by 1 % chitosan–EDTA. Furthermore, the complex was more bioadhesive than chitosan itself. The authors consider any zinc-containing enzyme to be susceptible to the effects of the complex. While this work was done with respect to intestinal enzymes, the present authors postulate that it is likely that this complex would be effective in the oral cavity in relation to enzymes needing zinc as a cofactor.

In some instances, the enzyme inhibitor has multiple effects. Glutathione served as a permeability enhancer and enzyme inhibitor in the delivery of pituitary adenylate cyclase-activating polypeptide via the buccal mucosa for type II diabetes [61]. Chitosan modified to contain thiol groups has not only increased mucoadhesion but has also been shown to improve enzyme inhibitory effects [61]. Bernkop-Schnurch et al. [62] reviewed several types of compounds, including

chitosan, polycarbophil, carboxymethylcellulose, polyacrylic acid, and alginate that have been thiolated. These thiomers have stronger mucoadhesive properties, probably due to the formation of disulfide bonds with cysteine-rich subdomains of mucus glycoproteins. The stronger bond not only allows better mucoadhesion but also improves enzyme inhibition and permeation enhancement. The enzyme inhibition seen with *H. gibbosa* [55] may similarly reflect, at least in part, the fact that the *Hakea* polymer contains sulfated sugars [63].

3.8 Choice of Chemical Enhancer

The choice of enhancer is related to the nature of the absorption problem. If the drug exhibits an inherently low permeability through the buccal mucosa, a permeation enhancer should be used. The selection of permeation enhancer is dependent on whether the drug is absorbed through the intercellular or transcellular route: Some permeation enhancers are thought to open the gap junctions and, thereby, enhance paracellular absorption; other enhancers interact with lipids and increase the fluidity of the cell membrane, thereby enhancing transcellular permeation.

If the drug has an inherently slow permeation through the mucosa, and a slow but prolonged absorption is desired (or is sufficient), a mucoadhesive may suffice to keep the dosage form in a suitable position for long enough to allow adequate absorption to occur. If a faster absorption is required, it is possible to incorporate both a permeation enhancer and a mucoadhesive.

If the absorption problem is that of a relatively slowly absorbed drug that is degraded during retention in the oral cavity or during passage through the mucosa, a substance that suppresses the metabolism of that class of drug is useful. If the drug in question is a peptide, it may be important to understand the location of the proteolytic activity, i.e., whether it is cytostolic, membrane bound, or within the intercellular space. The previously used technique of incubating the drug with the homogenate to determine if it serves as a substrate for enzymatic activity may be less useful, in the light of the knowledge that peptides usually permeate via the paracellular route. The exact location of the enzyme's activity is needed for a reasonable estimate of the metabolic breakdown of the drug. This determination may be undertaken when making an assessment of the feasibility of developing an oral mucosal system to deliver the drug. The type of protease present is equally important. To what extent the peptide is permeable via the transcellular route, may be an important question since this fraction is subject to metabolism by cytosolic enzymes.

If a peptide is being developed for buccal administration, the developer must ask: Which peptidases metabolize this drug? Carboxypeptidases are contained within the cytosol and will likely have no effect on the passage of paracellularly transported peptides. Endopeptidases are contained both within the cell and on the surface of the mucosa. According to Lee and Yamamoto [64], most of the peptidase activity (more than 80%) is cytosolic. Hence, the sensitivity, or the rate of

metabolism, of the peptide by endopeptidases is a parameter that the developer may want to quantitatively determine.

The most basic methods for assessment of permeation enhancers are in vitro permeation studies using cell culture or excised mucosa (see Chap. 6 for details). In a series of experiments with different permeation enhancers, for example, these methods can provide a rank-order correlation. They, usually, cannot be expected to accurately assess the magnitude of in vivo absorption via the human mucosa.

Since one of the major objectives of buccal and sublingual drug delivery is the replacement of an injection with a less intrusive method of delivery, the best possible outcome is achieved when the attained drug levels in humans mimic those of an injection. If the rate and extent of absorption is less than an injection, it may, nevertheless be possible to create a useful dosage form, based on the lower exposure. The developer of the product may, also, have to contend with more intersubject and intrasubject variability.

If the choice of absorption enhancer, based on the nature of the absorption problem, appears to be simple, it is an example of how the dissection and systematization of a problem may lead to an oversimplification. The exact nature of the absorption problem may not be readily ascertained. Successive in vitro and in vivo experiments may have to be conducted to determine the reasons for poor oral transmucosal absorption. The type of enhancer needed may not be clear when there are multiple issues with one drug. Even when an enhancer works, it may not be totally clear which aspect of a multifunctional enhancer's action is the most relevant. However, a systematic approach may, over time, lead to approaches in the choice of enhancers that are more appropriate and specific to the problem at hand. Similarly, an understanding of these phenomena may stimulate a search for newer and better absorption enhancers.

3.9 Conclusion

A systematic approach to the selection of absorption enhancers will, usually, lead to more effective choices. With widespread use of such an approach, a clearer understanding of the selection process will emerge. In addition, this may lead to the discovery or synthesis of improved enhancers. A practical problem is the regulatory approval process: In the USA, a new excipient is treated like a new drug in that toxicological studies, and other safety experiments, have to be performed. Once an excipient has been used in one approved product, it may then be used relatively easily, from a regulatory perspective, in additional products. This hurdle explains why there have been few introductions of new excipients of any type. The first company to introduce a new excipient faces the regulatory challenges. The gum from *H. gibbosa* may be one such excipient in need of a chaperone to guide it through the approval process. How many other such natural materials, or their synthetic cousins, are potentially available for use as enhancers?

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Chapter 4
Mucoadhesive Polymers as Enabling Excipients for Oral Mucosal Drug Delivery

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4.1 Introduction

Mucoadhesion and mucoadhesive dosage forms have attracted and attract considerable interest as a means for providing intimate contact between drug/delivery systems and the site of absorption and for prolonging residence time at the target site. Their capability to adhere to mucosal substrates (mucus layer which covers the epithelial tissues) is a unique feature of mucoadhesive polymers. Such materials are capable not only of prolonging the residence time of drug/delivery systems at the application/absorption site but also of modulating drug release. For these reasons, they are suitable for local disease treatment as well as for systemic drug availability improvement. The intimate contact with the absorption site typical of mucoadhesive polymers can also prevent drug degradation due to enzymes present in the lumen of the target mucosal site.

Academic and industrial research groups have performed extensive work within the last two decades to increase the knowledge of mucoadhesive polymer properties. These studies have led to a better comprehension of the mechanisms of mucoadhesion and consequently of the functional characteristics which render a polymer mucoadhesive. This understanding may lead to the development of new adhesive materials, possibly characterized by other functional properties.

The increased demand for macromolecule (proteins/peptides and oligonucleotides) delivery systems has increased the interest in this area [1]. The aim of the present chapter is to illustrate the meaning and the mechanisms of mucoadhesion, the theories developed to explain such a phenomenon and the mucoadhesive polymers most frequently employed as enabling excipients for oral mucosal drug delivery. In this respect, the functional characterization of these polymers are discussed, focusing on the physico-chemical properties involved in the mucoadhesion process, and specific case studies of oral mucoadhesion are described.

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A part of the chapter pays attention to materials characterized by multifunctional properties (namely, mucoadhesive and penetration enhancement properties), capable of enhancing drug absorption through the oral mucosa, thus playing a synergic role in improving system performance.

Finally, the tests employed for the evaluation of mucoadhesive properties, enabling the choice of the optimal mucoadhesive polymer for a given formulation, are discussed.

4.2 Bioadhesion and Mucoadhesion

4.2.1 Definitions

The term bioadhesion is defined as the state in which two biological surfaces or a biological surface and a synthetic surface are held together for an extended period of time by means of interfacial forces [2–6]. In pharmaceutical sciences, when the phenomenon of adhesion is associated with a biological surface covered by a mucus layer, the proper term is mucoadhesion: which represents the attachment of a natural or synthetic macromolecule to mucus and/or to an epithelial surface.

4.2.2 Mucus and Mucosae

The mucus is a gel layer adhered to the mucosae from which it is secreted. It acts as a lubrication layer and maintains the water balance between the lumen and the epithelium, and it also influences the immune response; furthermore, the mucus layer mediates the interaction between the environments and the epithelial cells [7]. Mucus is a water dispersion whose main components are mucin glycoproteins and lipids (0.5–5 %), inorganic salts (electrolytes; 0.5–1 %) and free proteins (1 %). The water content is approximately 95 %. The composition may vary depending on the origin and the role of mucus and on the health status of the individual. The mucins are a family of glycoproteins characterized by a molecular weight (MW) ranging from 1.000 to 40.000 kDa. Mucins possess a protein backbone (with a high content of serine and threonine) with side chains of oligosaccharides. The oligosaccharides account for 50–80 % of the mucin dry weight. The oligosaccharide chains are composed of glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine and fucose, further branched by means of S-links with ester sulphate and sialic acid moieties, eventually being O-acetylated [7]. The terminal groups of the oligosaccharide chains are often fucose, sulphate ester of galactose, *N*-acetylglucosamine and sialic acid. Sialic acid residue is negatively charged in physiological conditions: This confers negative charges to mucin at pH values higher than 2–3 [8]. Mucins may be classified into two groups: soluble secretory mucins and membrane-bound

mucins. The secretory mucins are secreted by mucosal absorptive epithelia or goblet cells and constitute a protective diffusion barrier for the underlying epithelia. The membrane-bound mucins possess a hydrophobic domain, integrated in the cell membrane surface.

4.2.3 Mechanisms of Mucoadhesion

The formation of the mucoadhesive joint, i.e. a bond between a mucoadhesive material and a mucous membrane, requires three successive steps [4, 6]:

- Contact stage: intimate contact between the mucoadhesive (polymer) and the mucous membrane.
- Interpenetration stage: interdiffusion of the polymer chains into the mucus layer to extend the contact area.
- Consolidation stage: formation of mechanical and/or chemical interactions responsible for the consolidation and strengthening of the mucoadhesive joint, which in turn results in a prolonged adhesion.

The first step is affected by the physical state of the materials involved, in particular by the hydration state. The contact stage is triggered by an intimate contact between the mucoadhesive and the mucosal epithelium. Such contact can be achieved by placing or holding a mucoadhesive material in direct contact with a mucosal surface (oral cavity, eye, vagina), by administering the mucoadhesive in particulate form in the respiratory tract or by adsorbing the mucoadhesive onto the mucosal surface of the gastrointestinal tract. In the case of semisolid or liquid dosage forms, the wetting and/or the spreading of the materials increase the contact area and favour the first step of the process. In the case of dry or partially hydrated mucoadhesive materials, the contact occurs as a consequence of wetting, hydration and swelling. The contact causes a reduction in surface free energy, the loss of two distinct surfaces and the formation of a new interface.

At this point, the second step occurs: the interpenetration of the polymer chains into the mucus layer causes chain entanglements.

In the consolidation stage, chain entanglement causes the formation of mechanical and chemical bonds, thus contributing to the strengthening of the mucoadhesive joint.

The mechanical bonds are physical connections playing a role at the mucoadhesive interface. Macroscopically, they correspond to a penetration of mucoadhesive polymer into the mucus layer; microscopically, they involve the physical entanglements of polymer chains into the mucus layer [9]. The rate of penetration of the polymer into the mucus layer depends on the peculiar properties of the polymer such as chain flexibility and length.

The chemical bonds include strong primary bonds as well as weak secondary forces [2, 4–6]. Depending on the chemical structure of the polymer, the chemical bonds involved in the formation of mucoadhesive joint can be:

- Ionic bonds: electrostatic interactions between two oppositely charged substances resulting in a strong bond.
- Covalent bonds: due to electrons shared between the bond atoms to fill the orbitals.
- Hydrogen bonds: attractions between a hydrogen atom, covalently bonded with an electronegative atom (oxygen or nitrogen) and slightly positively charged, with another electronegative atom slightly negatively charged.
- van der Waals bonds: weak interactions occurring between dipole–dipole and dipole-induced dipole.
- Hydrophobic bonds: interactions occurring in aqueous media between hydrophobic groups, due to the association of non-polar groups to minimize the increase in entropy.

The mucoadhesive joint is reversible: its failure usually takes place at the interface between the adhesive and mucus layers, which is the weakest region involved [4, 9]. The water transport from the tissue to the adhesive affects the residence time in two opposite ways: it weakens the adhesive (polymer) by dilution and simultaneously increases mucus cohesion by dehydration.

4.2.4 Theories for Mucoadhesion

Different theories were developed to explain the mucoadhesive phenomenon; the most commonly invoked are: electronic, adsorption, mechanical, diffusion, wetting and fracture theories [4–6, 9, 10].

The electronic theory assumes that an electron transfer occurs between the adhesive and the mucus layer as a result of the differences in their electronic structures, with the formation of an electrical double layer at the adhesive–mucus interface. The electron transfer across this electrical double layer determines attractive forces and thus mucoadhesion [11].

The adsorption theory explains mucoadhesion in terms of secondary surface forces such as van der Waals, hydrogen bonds and hydrophobic interactions. This theory explains the mucoadhesive properties of some hydrophobic materials with poor chemical affinity for the hydrophilic mucus layer [12, 13].

The mechanical theory assumes that adhesion is due to an interlocking of the adhesive into the irregularities of a rough surface. Such a surface increases the contact area between the adhesive and the mucus providing a wider place available for the interaction [6].

The diffusion theory explains mucoadhesion with a diffusion of the adhesive into the mucus layer to a depth sufficient to create a semi-permanent adhesive layer. This process is influenced by the MW (polymer chain length) of the adhesive as well as by its diffusion coefficient which determines the concentration gradient and conse-

quently the layer depth. The good solubility of the adhesive into the mucus layer is a further feature that affects mucoadhesion [14].

The wetting theory, developed for liquid adhesives, considers the interfacial energy of the mucus layer and the adhesive. The key factor is the capability of the adhesive to spontaneously spread on a surface, which in turn is influenced by its affinity for the mucus layer [15].

The fracture theory considers the force required for the separation of the mucoadhesive joint. The fracture is considered equal to the adhesive force and is assumed to occur at the mucoadhesive interface [16].

None of these theories can explain mucoadhesion on its own: a combination of theories provides an exhaustive description of the process. Moreover, the applicability of the different theories depends on the physico-chemical properties of the adhesive considered and of the biological substrate (depth of mucus and continuity of the mucus layer).

4.2.5 Factors Influencing Mucoadhesion

Mucoadhesion is influenced by both the intrinsic properties of the adhesive material and the environmental conditions where mucoadhesion occurs [5].

When mucoadhesion is investigated by in vitro or *ex vivo* methods, attention has to be devoted to the type and complexity of the biological substrate employed: purified or partially purified mucin, type of mucin and type of mucosa [9].

A given mucoadhesive may express different mucoadhesive properties depending on the biological substrate properties. The keratinisation degree and the carbohydrate moieties expressed by the mucosal surface are key factors in this perspective. The roughness and the hydration state of the biological substrate may also influence mucoadhesion [17].

The mucus turnover also has to be considered in view of the in vitro/*ex vivo* correlation. Moreover, the technique employed to measure mucoadhesion may be crucial to determine the mucoadhesive potential.

The characteristics of polymers most relevant to mucoadhesive properties have been determined by screening many polymers that were known to be mucoadhesive.

The adhesive-related factors depend on the chemical and physical properties of the molecule: In particular, the presence of functional groups able to form hydrogen bonds and the charge density, the chain flexibility, the MW and the concentration are the key factors.

The presence of carboxylic groups favours the establishment of ionic interactions and hydrogen bonds between the mucin and the polymer chains. The hydroxyl, amino and sulphate groups are also likely to take part in the formation of the mucoadhesive joints by means of hydrogen bonds and/or charge–charge interactions depending on the environmental pH [18]. The charge density, i.e. the amount of charged groups per polymer chain, influences the extent of ionic interactions with the mucin macromolecules, and it is also related to the medium pH and to the polymer functional groups [18].

The chain flexibility of a polymer is a critical point for chain entanglements with mucin macromolecules and consequently for the interpenetration and the diffusion into the mucus layer. If the adhesive is cross-linked, the chain mobility is reduced. Moreover, chains can penetrate into the mucus, thus reducing the mucoadhesive strength.

The MW of the adhesive is also a crucial feature. The optimum weight to maximize mucoadhesion is thought to be 100 kDa for linear chains: in fact, interpenetration and chain diffusion are more effective when the adhesive chains are limited in length, whereas entanglement plays a major role for high MW adhesives. Mucoadhesion of branched structures is not directly related with branching degree, but depends on the tridimensional conformation of the molecule. For example, in the case of high MW (1.900 kDa) dextran, characterized by helical conformation, all the functional groups involved in the mucoadhesive joint are shielded, resulting in poor mucoadhesive properties [19].

Polymer concentration also affects mucoadhesion. The increase in concentration favours polymer cohesion: An increase above the isotonic concentration promotes water transport from the biological substrate to the polymer, hence strengthening the mucus layer. This also affects the extent of the interpenetration and the intensity of the mucoadhesive joint.

4.3 Oral Mucosal Drug Delivery

The oral cavity has been used as a site for local and systemic drug delivery [20–22].

Oral mucosal drug delivery is subdivided into buccal and sublingual routes.

The buccal route is widely applicable for drug administration of mucoadhesive systems intended for either a local or a systemic action, whereas the sublingual route is mostly useful for fastest onset of a systemic effect as in the case of *angina pectoris*.

The buccal mucosa lines the inner cheeks, and buccal formulations are usually placed in the mouth between the upper gingivae and cheek. Although less permeable than the sublingual area, the buccal mucosa is well vascularized, and drugs can be rapidly absorbed into the venous system underneath such mucosa.

This renders the buccal route of particular interest for the systemic absorption of typically large drug molecules (hydrophilic and unstable proteins, oligonucleotides and polysaccharides), as well as conventional small drug molecules.

The advantages of oral mucosal drug delivery can be summarized in: (a) bypass of the gastrointestinal tract and hepatic portal system, increasing the bioavailability of orally administered drugs that otherwise undergo hepatic first-pass metabolism; in addition, the drug avoids degradation due to pH and digestive enzymes of the middle gastrointestinal tract; (b) ease of drug administration and improved patient compliance due to the elimination of pain associated with injections; administration of drugs in unconscious or incapacitated patients; convenience of administration as compared to injections or oral medications; (c) prolonged drug delivery; (d) faster

onset and decline of delivery due to the absence of stratum corneum, with consequently lower intersubject variability in comparison to transdermal patches; and (e) relatively rapid onset of action and possibility to remove the formulation if the therapy must be discontinued.

On the other hand, the drawbacks of oral mucosal drug delivery are: (a) physiological removal from the oral cavity (washing effect of saliva and mechanical stress), which takes the formulation away from the mucosa, resulting in a very short exposure time and unpredictable distribution of the drug on the action/absorption site; (b) the need to ensure patient compliance in terms of taste, irritancy and "mouth feel"; and (c) for systemic delivery, the relative impermeability of oral mucosa to drug absorption, especially for large hydrophilic biopharmaceuticals, is of major concern.

4.4 Formulation Design for Buccal Delivery

As previously discussed, for mucosal administration, conventional dosage forms are not able to assure therapeutic drug levels in the mucosa and systemic circulation. To obtain the therapeutic action, it is necessary to prolong and improve the contact between the active substance and the mucosa; therefore, the formulations intended for buccal administration should ideally contain: penetration enhancers, to improve drug permeation across mucosa (transmucosal delivery) or into deepest layers of the epithelium (mucosal delivery); enzyme inhibitors, to protect the drug from degradation by mucosal enzymes and solubility modifiers to enhance solubility of poorly soluble drugs; and typically mucoadhesive agents, to maintain an intimate and prolonged contact of the formulation with the absorption site.

4.4.1 Penetration Enhancers

In order to design penetration enhancers, with improved efficacy and a reduced toxicity profile, it is required to understand the relationship between enhancer structure and the effect induced in the membrane and the mechanism of action. However, selection of enhancer and its efficacy depends on the physico-chemical properties of the drug, nature of the vehicle and other excipients which are drug specific and should be safe and non-toxic, pharmacologically and chemically inert, non-irritant and non-allergenic. Various compounds have been investigated for their use as buccal penetration enhancers in order to increase the flux of drugs through the mucosa: surfactants (anionic, cationic, non-ionic), bile salts (e.g. sodium glycol deoxycholate, sodium glycocholate, sodium taurodeoxycholate, sodium taurocholate), fatty acids (e.g. oleic acid, caprylic acid, lauric acid, lysophosphatidylcholine, phosphatidylcholine), cyclodextrins (α, β, γ, cyclodextrin, methylated β-cyclodextrins), chelators (e.g. ethylenediaminetetraacetic acid (EDTA), citric

acid, sodium salicylate, methoxy salicylates), cationic compounds (poly-L-arginine, L-lysine) and positively charged polymers (chitosan, trimethylchitosan). The mechanism of permeation enhancement can be summarized as changing mucus rheology, increase in the fluidity of lipid bilayer membrane, action on the components at tight junctions, overcoming the enzymatic barrier and increase in the thermodynamic activity of drugs [23, 24].

4.4.2 Enzyme Inhibitors

Co-administration of a drug with enzyme inhibitors is another strategy to improve buccal absorption, particularly of peptides. Enzyme inhibitors, such as aprotinin, bestatin, puromycin and some bile salts stabilise protein drugs by different mechanisms, including change in the activities of enzymes, altering the conformation of the peptides or proteins and/or rendering the drug less accessible to enzymatic degradation [25]. In addition, some mucoadhesive polymers, such as polyacrylic acid and chitosan derivatives, have been proved to inhibit enzyme activity [26, 27]. In particular, polyacrylic acid (carbomer) is able to bind the essential enzyme cofactors such as calcium and zinc and, by a change in conformation, can cause enzyme autolysis and loss of enzyme activity. Moreover, the chemical modification of chitosan (cationic polymer) with EDTA produces polymer conjugate chitosan–EDTA that is a very potent inhibitor of metallopeptidases, such as carboxypeptidase [27]. In recent years, polymer derivatization with thiol groups on poly(acrylates) or chitosans has been demonstrated to improve polymer enzyme inhibitory properties [28].

4.4.3 Solubility Modifiers

In spite of the increase in bioavailability of hepatically metabolized drugs by buccal delivery, the poor solubility of drug in the saliva may impede drug release from its device for uptake by the buccal mucosa. Solubilization of poorly water-soluble drugs by complexation with cyclodextrins and delivery via the buccal mucosa is advantageous in increasing drug absorption and bioavailability. It has been reported that the release of felodipine from buccal tablets comprising hydroxypropyl-β-cyclodextrin–felodipine complex and hydroxylpropylmethyl cellulose (HPMC) is characterized by a complete and sustained release of the drug which is associated with an enhanced buccal permeation. These results could be attributed to the ability of hydroxypropyl-β-cyclodextrin to form a complex with felodipine, resulting in an increase in apparent drug solubility, dissolution rate and permeability [29]. The results demonstrate that these polymeric formulations with inclusion complexes afford high utility as a transmucosal drug delivery system for a complete and sustained drug release with enhanced permeability. Imidazole antimycotics (e.g. miconazole, clotrimazole) are extensively used in the local treatment of fungal infections in the

oral cavity. Due to their low water solubilities and high lipophilicities, they were released extremely slowly from the lipophilic chewing gum bases. Formulating the hydroxypropyl-β-cyclodextrin inclusion complex of these antimycotics into chewing gums was found to increase the drug release [30].

4.5 Mucoadhesive Polymers and Their Application in Oral Mucosal Drug Delivery

Mucoadhesive polymers used in the oral cavity can be classified as non-specific bioadhesives, called first-generation bioadhesives, and novel second-generation mucoadhesive polymers [24, 31, 32].

4.5.1 First-Generation Mucoadhesive Polymers Used in the Oral Cavity

First-generation polymers can be natural or synthetic, water-soluble or water-insoluble, charged or uncharged polymers. Examples of recent bioadhesive buccal polymers are listed in Table 4.1 [24, 32].

Table 4.1 Mucoadhesive polymers employed for oral mucosal drug delivery. (Modified from [24])

Criterion	Type	Polymer
Source	Natural	Chitosan, hyaluronic acid
		Agarose, gelatin, sodium alginate
		Various gums (guar, *Hakea,* xanthan, gellan, carrageenan, pectin and sodium alginate)
	Semi-synthetic	Cellulose derivatives
		(CMC, thiolated CMC, sodium CMC, HEC, HPC, HPMC, MC, methylhydroxyethyl cellulose)
	Synthetic	Poly(acrylic acid)-based polymers
		(CP, PC, PAA, copolymer of acrylic acid and PEG)
		PHPMm, poly(ethylene oxide), PVA, PVP, thiolated polymers
Aqueous solubility	Water soluble	CP, HEC, HPC (water temperature range: <0–38 °C), HPMC (cold water), PAA, sodium CMC, sodium alginate, PVP, MC, sodium CMC
	Water insoluble	Chitosan (soluble in dilute aqueous acids), EC, PC

Table 4.1 (continued)

Criterion	Type	Polymer
Charge	Cationic	Chitosan, trimethylated chitosan, aminodextran, dimethylaminoethyl (DEAE)-dextran
	Anionic	Chitosan–EDTA, CMC, pectin, PAA, PC, sodium alginate, sodium CMC, xanthan gum, CP and polyacrylate cross-linked modifications
	Non-ionic	Hydroxyethyl starch, HPC, poly(ethylene oxide), PVA, PVP, scleroglucan, Eudragit-NE 30D
Potential bioadhesive forces	Covalent	Cyanoacrylate
	Hydrogen bond	Acrylates (hydroxylated methacrylate, poly(methacrylic acid)), CP, PC, PVA
	Electrostatic interaction	Chitosan

CMC carboxymethylcellulose, *CP* Carbopol, *EC* ethyl cellulose, *HEC* hydroxyethyl cellulose, *HPC* hydroxypropyl cellulose, *HPMC* hydroxypropylmethyl cellulose, *MC* methyl cellulose, *PAA* poly(acrylic acid), *PC* polycarbophil, *PEG* poly(ethylene glycol), *PHPMAm* poly(*N*-2-hydroxypropyl methacrylamide), *PVA* polyvinyl alcohol, *PVP* polyvinylpyrrolidone

The duration of bioadhesion is largely determined by the fast turnover of the mucus layer [33]. Factors such as saliva secretion, food intake, local pH and compositions of delivery systems also strongly affect mucoadhesion.

In the remainder of this chapter, examples of natural (cationic, chitosan and chitosan derivatives, and anionic, hyaluronic acid), semi-synthetic (cellulose derivatives) and synthetic polymers (poly(acrylic acid)-based polymers) that have been fruitfully employed for oral mucosal drug delivery are reviewed. The choice of natural polymers is justified by their multifunctional behaviour: In fact, besides being mucoadhesive, they also possess penetration enhancement properties.

4.5.1.1 Chitosan

Physico-chemical Properties Chitosan is a polysaccharide derived from chitin by means of a deacetylation reaction. Chitin is the main component of the exoskeleton of crustaceans, insects and fungi. Chitosan (Fig. 4.1) is a linear copolymer of glucosamine and *N*-acetylglucosamine (β-[1→4]-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose).

The structure of chitosan is very similar to that of cellulose (characterized by (1–4)-linked D-glucose units), with hydroxyl groups in the C2 positions of the glucose rings.

The term chitosan is used to describe a series of polymers with different deacetylation degrees (DD; that is as the percentage of primary amino groups in the polymer backbone) and average MWs [34]. The DD of chitosan is usually between 70 and 95 %, and the MW between 10 and 1000 kDa.

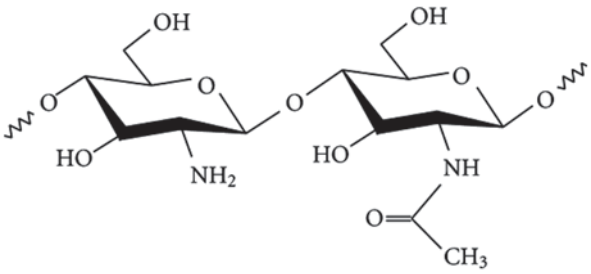


Fig. 4.1 Chemical structure of chitosan

Chitosan is polycationic and soluble in acidic environment, and slightly soluble in neutral and weak alkaline environments: its cationic character is quite unusual in natural polysaccharides, which renders chitosan an interesting material.

The DD affects chitosan solubility in aqueous solutions: lowering the DD increases the solubility.

Since the D-glucosamine unit is characterized by a pK_a value of 7.5 [34], the basic nature of chitosan depends on DD: the chitosan pK_a value is around 6.5. This implies that chitosan, as a free base, dissolves slowly in acidic and slightly acidic aqueous solutions, whereas it precipitates from solution in the form of a free base when the pH increases up to neutrality.

Low MW chitosans (MW approximately 10 kDa or below) and chitosan salts (for example, hydrochloride) may be more readily soluble in water.

In acidic and slightly acidic media, chitosan spontaneously gelifies, upon water absorption. A decrease in medium pH increases viscosity, conceivably owing to the more extended chain conformation (instead of the random coil) at low pH values, caused by repulsive forces between positively charged amino groups [35]. Moreover, the increase in DD and consequently in the polyelectrolyte behaviour increases the viscosity of chitosan solutions. Chitosan solutions exhibit pseudoplastic flow: the viscosity decreases as shear force increases [36].

Mechanism of Mucoadhesion The hydrophilic properties of chitosan cause "adhesion by hydration" (the simplest mechanism of adhesion) with adherence of chitosan solution to mucosal surfaces. In particular, chitosan possesses the capability to attract water from the mucus gel layer in contact with the epithelial surface [37].

Mucoadhesion of chitosan was first studied by Lehr et al. [38], who reported that many commercially available chitosans adhere fairly strongly in vitro. In these studies, the forces required to detach chitosan films from isolated porcine intestinal segments were measured. One important finding of this study was that the adhesive properties of chitosans persisted during repeated contacts with the biological substrate even though chitosan was in a swollen/hydrated state. This suggested that not only adhesion by hydration was involved but also additional mechanisms, such as hydrogen bonds and ionic interactions. According to the authors, the most important mechanism of action of chitosan is the ionic interactions between the positively charged amino groups of the polymer and the negatively charged sialic acid residues of the mucus gel layer.

Further studies regarding the interactions between chitosan and mucus [39, 40] demonstrated that the primary mechanism of mucoadhesion at the molecular level is the formation of electrostatic bonds [41]. It has also been shown that adsorption of hydrophilic chitosan molecules on mucosal surfaces and mucus dehydration are involved in the mucoadhesion properties of chitosan [42].

Moreover, the interactions of chitosan with mucus and its mucoadhesive properties are affected by both physiological factors and physico-chemical properties of the polymer.

The extent of mucin adsorption by chitosan increases on increasing the sialic acid residues [40]. In fact, Deacon et al. [41] showed that interactions are more pronounced with mucins from the cardiac region of the porcine stomach than from those of the corpus or antrum. This finding could be explained by the composition of secretions of the gastric cardiac region (mucin chains rich in sialic acid residues) [43]. Since the amounts of sialic acid in mucosal secretions vary, the force of adhesion of chitosan to mucus may be different depending on the mucosae considered. Moreover, at high pH values, chitosan molecules are more entangled, whereas as pH decreases the molecules become more ionised, are uncoiled, possess high charge density and assume a more elongated shape. Hence, at low pH values, chitosan has better chances for an intimate contact with the epithelial membrane and for the electrostatic interaction with the anionic component (sialic acid) of the glycoproteins of the epithelial cells [40, 43].

The MW and charge of chitosan are also important for mucoadhesion: the increase in MW results in stronger adhesion [44]. This feature is due to the deeper interpenetration of polymer and mucus chains favoured by the chain length of the polymer [45]. The interpenetration determines a chain interlocking: the conformational changes and the chemical interactions between chitosan and mucin are likely to modify their rheological behaviour. The changes in viscosity and viscoelastic properties of fully hydrated chitosan and mucin dispersions are considered a proof of the formation of mucoadhesive interactions. In particular, a measure of the strength of the mucoadhesive joint can be derived from the increase in viscosity and viscoelastic properties (rheological synergism) of the polymer when mixed with mucin [46].

At high polymer to mucin weight ratio, a minimum in viscosity occurs, whereas in the presence of a mucin excess a synergistic increase in viscosity is observed: Such an increase indicates the formation of a mucoadhesive joint [46].

It has been demonstrated that the increase in charge density determines an improvement of the adhesive properties of chitosan. Since charge density is affected by DD and by cross-linking, the extent of mucoadhesion is directly related to the number of free amino groups on chitosan. At the same time, the greater the positive charge density, the stronger the mucoadhesive joint [40, 47, 48]. These findings suggest that the adhesive properties of chitosan should improve on increasing the DD and on decreasing the cross-linking degree.

The mucoadhesive properties of chitosan were at first assessed in vitro, in isolated mucosal preparations. Chitosan has been found to adhere to isolated porcine gastric [49] and intestinal [44] mucosae upon static contact between the polymer

and the biological substrates. Chitosan mucoadhesive properties were assessed in vitro in wash-off experiments using rat intestine as biological substrate [40, 50, 51].

Results concerning the mucoadhesion of chitosan formulations in vivo refer to the adhesion of chitosan microspheres to murine gastric mucosa [52] and rat intestinal mucosa [53]. In these studies, the stomach and small intestine of anesthetized animals were excised at a predetermined time after administration of the formulations containing chitosan and a fluorescent marker. The fluorescence of the various regions of the gastrointestinal tract enables researchers to trace the adhesion of the formulations. In the study of Remuñan-López et al. [52], chitosan was found in the mouse stomach 4 days after administration, even though the kinetics of adhesion and the amount of polymer plus fluorescent label were not investigated.

Shimoda et al. [53] demonstrated the adhesion of chitosan microspheres to rat intestinal mucosa. Different amounts of microspheres were retained in the intestine over a period of 8 h. Although the chitosan formulations examined exhibited mucoadhesive properties in rodents, the authors did not discuss whether their results might be reproducible in humans. It is, however, well known that the extrapolation to man of results obtained in animal studies carried out with formulations intended to be retained in the stomach is subjected to substantial limitations. Although chitosan exhibited good adhesive properties in rats, adhesion was rare in human volunteers. Gamma scintigraphic investigations showed no significant differences between gastric residence times of chitosan and residence time of a non-mucoadhesive reference material (lactose) administered in man. It was concluded that the poor correlation of results obtained in rodents and in mammalians (dog, man) can mostly be explained on the basis of the physiological differences between the two species [54].

These results demonstrate that more information on the in vivo behaviour of systems containing chitosan is needed, and that more attention must be paid to the behaviour of chitosan formulations in humans.

To enable the optimization of chitosan-based drug delivery systems, the effects of different variables, e.g. chitosan content and chitosan grade, on drug absorption and/or residence times of formulations are to be studied. The effects of the physicochemical properties of chitosan also require systematic evaluation, in vitro and in vivo.

Despite the notable research on chitosan carried out in recent decades, many questions remain unanswered.

More information is needed about the effect of chitosan grade on the properties of pharmaceutical formulations. Unfortunately, the commercial grades of chitosan are not always well characterized: this drawback has limited the use of this polymer [55]. The comparison of results obtained by different research groups is rather difficult since the properties of the chitosans studied, in particular the DD and/or the MW, are not specified. Only recently, attention has been paid to the production of chitosans characterized by particular physico-chemical properties and desired DD and MW.

The major gap of studies on chitosan is the evaluation of in vivo behaviour of chitosan-based formulations especially in humans. Further in vivo studies would

reveal the value of chitosan as a pharmaceutical excipient, and allow products containing chitosan to be marketed.

Chitosan is also well known as a penetration enhancer towards monostratified or pluristratified epithelia. In particular, chitosan enhances drug absorption through mucosae monostratified and endowed with tight junctions such as the intestinal [56–58] and the nasal [59, 60] mucosae. It has been shown that chitosan can improve drug permeation across pluristratified and lacking tight junctions epithelia such as buccal [36, 61–63] and vaginal mucosae [64]. Finally, chitosans can increase drug absorption through pluristratified epithelia, characterized by tight junctions, such as the corneal epithelium [65–67].

As for the mechanism of penetration enhancement, it is generally thought that chitosan disrupts intercellular tight junctions [57] and it is also able to reorganize the desmosomal junctional proteins [63].

Pharmaceutical Applications in Oral Mucosal Drug Delivery Chitosan has received considerable attention as a possible pharmaceutical excipient in recent decades. It has been evaluated as an excipient in conventional formulations and controlled release drug delivery systems, intended for mucosal and transmucosal administration.

The potential value of chitosan as a novel excipient with extensive application in pharmaceutical products has been highlighted in several reports and review articles [55, 68–74].

Chitosan has been approved and included since the fourth edition of the *European Pharmacopoeia* (2002).

Several properties of chitosan make it valuable as a pharmaceutical excipient. Good biocompatibility and low toxicity of chitosan [75–78], as well as its abundant sources, are features that each new excipient should have.

Formulations based on chitosan can easily be prepared by conventional granulation and tabletting techniques. One feature that makes chitosan particularly interesting is its ability to hydrate and to form gels in acidic aqueous environments. Due to this gel-forming ability, chitosan has been used in slow-release drug delivery systems.

Chitosan has been evaluated in vitro as a drug carrier in hydrocolloids and gels [79, 80], and as a hydrophilic material retarding drug release in tablets [81, 82], granules [83, 84] and microparticles [85–87]. The hydrophilic nature of chitosan has also determined its use in immediate-release formulations, e.g. as a disintegrant in tablets, where it has been found to have effects similar to or better than those of microcrystalline cellulose [88–90], and as an excipient to increase the dissolution rate of poorly soluble drug substances [91, 92].

Chitosan has been widely employed in drug delivery systems intended for transmucosal and mucosal administration by various routes: ophthalmic, nasal, buccal, periodontal, gastrointestinal, vaginal and transdermal.

As for buccal delivery, chitosan has been employed to develop tablets and films in order to prolong the residence time of the formulations in the oral cavity [93]. Moreover, chitosan represents an excellent candidate for the treatment of

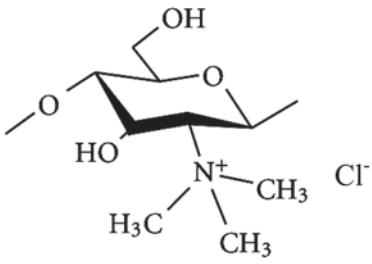


Fig. 4.2 Chemical structure of *N,N,N*-trimethyl chitosan chloride

oral mucositis. Its bioadhesive and antimicrobial properties result in the palliative effects of an occlusive dressing and also enable the delivery of drugs, such as antifungal agents against *Candida albicans* [93].

Local delivery of drugs and other bioactive agents into the periodontal pocket has received a lot of attention. Semisolid (gels) and solid (mini-matrix and film) based on chitosan were developed to deliver antimicrobial drugs into the periodontal pocket for local therapy of infections, aimed at improving efficacy and acceptability. Chitosan itself possesses antibacterial and antifungal activity due to electrostatic interactions between the amino groups of the polymer and the anionic sites (carboxylic acid residues and phospholipids) of bacterial walls [94].

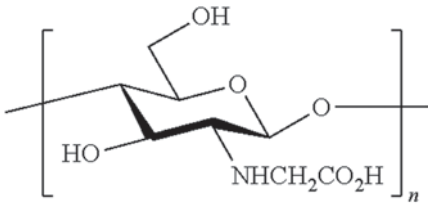
4.5.1.2 Chitosan Derivatives

A drawback to the use of chitosan is due to the fact that chitosan and chitosan salts are poorly soluble at pH values close to neutrality. Chitosan precipitates from solution when the pH of the solvent is above 6 or 6.5 depending on the DD of chitosan, as previously reported. Obviously, in those environments, chitosan is not effective.

To overcome this problem, various chitosan derivatives were synthesized to improve chitosan solubility.

N,N,N-Trimethyl Chitosan Chloride This polymer, shown in Fig. 4.2, is a chitosan derivative soluble in a broader pH range than chitosan. It is synthesized starting from chitosan by means of a trimethylation, quaternization of the chitosan amino group. The substitution of the primary amine units with methyl groups and the prevention of hydrogen bond formation between the amino and the hydroxylic groups of the chitosan backbone increase solubility.

Depending on steps and duration of the synthetic process, *N,N,N*-trimethyl chitosan chloride (TMC) can be quaternized at different degrees: the degree of substitution is expected to play an important role on the mucoadhesive properties of TMC. Sandri et al. reported that the mucoadhesive properties of TMC increased on increasing the quaternization degree [63]. On the contrary, Snyman et al. described a decrease in the maximum force of detachment on increasing the quaternization degree [95]. These results seem to be in disagreement: it is necessary to underline that Snyman et al. employed a mucoadhesive parameter not normalized on the basis



MCC

Fig. 4.3 Chemical structure of mono-carboxymethyl chitosan

of the intrinsic cohesive properties of polymer solutions. The use of the normalized mucoadhesive parameter allows for the elimination of the contribution of sample consistency in the evaluation of the mucoadhesive potential.

The penetration enhancement properties of TMCs at pH values close to neutrality increase on increasing the quaternization degree [56, 69, 70, 96, 97]: TMC with a low trimethylation degree failed while TMC with a trimethylation degree equal to 60% increased the permeability of model molecules across cell monolayers, thus indicating that a threshold value of charge density of the polymer is necessary to trigger the opening of tight junctions at neutral pH values.

The effect of TMC on Caco-2 cell monolayers was further investigated. By using fluorescent probes, impermeable to cell membrane and confocal laser scanning microscopy, it was visualised that TMC polymers widen the paracellular pathways without cell membrane damages [96].

Mono-Carboxymethyl Chitosan Another chitosan derivative synthesized to overcome the problem of chitosan solubility is mono-carboxymethyl chitosan (MCC; Fig. 4.3) [98].

In this derivative, the amino groups of chitosan are substituted with methylcarboxy acid. The N-substitution with alkyl groups increases the aqueous solubility of chitosan without affecting its cationic character; substitutions with moieties bearing carboxyl groups yielded polymers with polyampholytic properties.

Thanks to these properties, MCC is able to form clear gels or solutions (depending on polymer concentration) even in the presence of polyanionic compounds like heparin at neutral and alkaline pH values, whereas it aggregates at acidic pH. On the contrary, chitosan and TMC form complexes with polyanions that precipitate.

MCC showed the ability to prolong precorneal drug retention, due to its viscosity-increasing effect, its ability to bind ofloxacin and, probably, its mucoadhesive properties [97].

Moreover, MCC significantly increased the permeation of anionic macromolecules, showing that, even as polyampholyte, chitosan is able to induce the opening of tight junctions; nevertheless, it acts at concentrations higher than TMC [97].

5-Methylpyrrolidinone Chitosan 5-Methylpyrrolidinone chitosan (MPC; Fig. 4.4) is a chitosan derivative in which the amino groups are partially substituted with methylpyrrolidinone (a well-known skin penetration enhancer). Such a

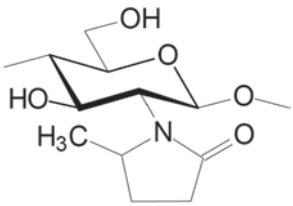


Fig. 4.4 Chemical structure of 5-methylpyrrolidinone chitosan

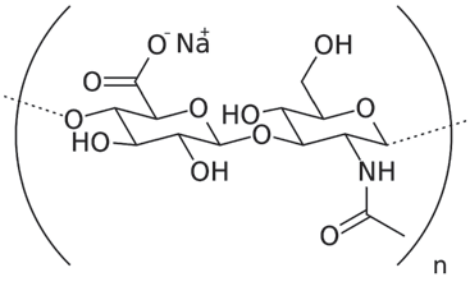


Fig. 4.5 Chemical structure of hyaluronate

derivatization does not improve solubility properties of chitosan and MPC is poorly soluble at pH close to neutrality. However, such a derivatization improved penetration enhancement properties especially in the case of low MW chitosan [62].

Even though the derivatization partially shields the cationic charges, MPC maintains the good mucoadhesive properties of chitosan.

4.5.1.3 Hyaluronic Acid and Derivatives

Physico-chemical Properties Hyaluronic acid (sodium hyaluronate and hyaluronan; Fig. 4.5) is a naturally occurring linear polysaccharide, glycosaminoglycan constituted by alternating residues of D-guluronic acid (carboxylic acid) and glucosamine linked in repeating units [99]. It is widely distributed in the extracellular matrix of connective tissues and it is present in the synovial fluids and in aqueous and vitreous humours of the eyes.

The physiological concentration and MW of hyaluronic acid determine its rheological properties of high viscosity at low shear rates and low viscosity at high shear rate, typical of pseudoplastic polymer solutions. These characteristics are fundamentally responsible for the structural functions in the connective tissue of the intercellular matrix and also for the locating and cushioning properties in synovial fluids and in the eye humours.

The reaction of hyaluronate carboxylic groups with alcohols, in the presence of an aprotic solvent, yields esters of hyaluronate with different physico-chemical properties compared to hyaluronate itself. Esters of hyaluronate with benzylic alcohol and ethanol have been synthesized and named HYAFF7 and HYAFF11, respectively.

Mechanism of Mucoadhesion Hyaluronic acid is characterized by good mucoadhesive properties toward rat small intestine [100]. The great number of COOH groups promotes adhesion to the biological substrates through hydrogen bond formation: the presence of unionised groups favours mucoadhesion [100].

When the polymer is in a hydrated state, a decrease in the MW of hyaluronic acid results in an increase in mucoadhesive properties which strictly depend, however, on the pH and buffer capability of the biological substrates [101].

The mucoadhesive potential is affected by the hydration rate and is dependent on the MW up to a certain cut-off value. Above this value, a further increase in MW does not produce an increase in the mucoadhesive potential [100]. Esterification of carboxylic groups leading to HYAFF decreases the mucoadhesive properties of hyaluronic acid, due to the lower disposition to form hydrogen bonds with the biological substrates, although HYAFF11 still possesses good mucoadhesion [102]. The mucoadhesive properties are also affected by hydrophilicity: the faster the hydration upon contact with the mucus, the faster mucoadhesion occurs [100].

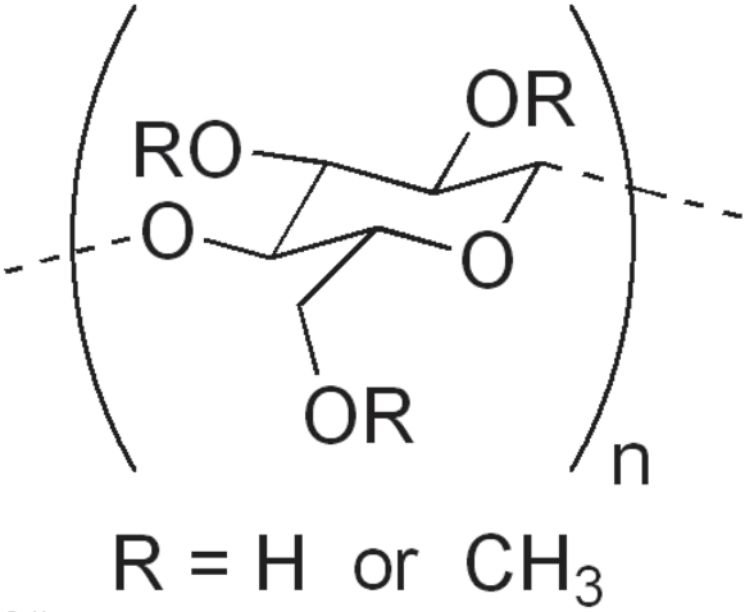
Pharmaceutical Applications in Oral Mucosal Drug Delivery Recently, the mucoadhesive properties of different grades of hyaluronic acid have been investigated to evaluate their suitability for the development of oral mucosal delivery systems to be used for the treatment of oral mucositis in cancer patients [103]. In most cases, pharmaceutical preparations based on hyaluronic acid used in the oral cavity are intended for the treatment of periodontitis [104, 105]. Despite the good mucoadhesive potential of the polymer [100], in such preparations, its biological, namely tissue repairing, properties are invoked.

4.5.1.4 Cellulose Derivatives

Physico-chemical Properties Cellulose (Fig. 4.6) is the starting polymer source for all cellulose derivatives and it is the most abundant polymeric naturally occurring material.

Structurally, cellulose consists of repeating units of anhydro-β-D-glucopyranose units linked by β-1,4-glycosidic bonds. The CH_2OH, OH and the glycosidic bonds are all equatorial with respect to the planes of the pyranose rings.

Cellulose is insoluble in water: the synthesis of derivatives primarily aims to prepare polymers characterized by better solubility properties in aqueous environments and also a pH-dependent solubility. Among the various cellulose derivatives, some alkyl cellulose ethers, in particular HPMC, sodium carboxymethyl cellulose (NaCMC), hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (HEC), are characterized by good water solubility [106]. The hydration rates depend on the chemical nature of the substituents and on the substitution degree. In general, the hydrophilicity of the cellulose ethers decreases with an increase in the alkyl chain length. The polymer solution viscosity increases with an increase in concentration and MW. It also changes with temperature decreasing when warmed and increasing when cooled. A temperature increase causes a weakening of polymer–water interaction (solvation) and consequently a decrease in viscosity. Such polymer so-



Cellulose	R=H
NaCMC	R=H, CH_2COONa-
HPMC	R=CH_3, $OCH_2CH(OH)CH_3$
HPC	R=H, $OCH_2CH(OH)CH_3$

Fig. 4.6 Chemical structures of cellulose derivatives

lutions exhibit a pseudoplastic behaviour: HEC and NaCMC solutions generally show thixotropic properties, while solutions of HPMC and HPC are characterized by little or no thixotropic flow [106]. Solutions of HPMC, HEC and HPC, due to their non-ionic nature, withstand pH changes and low ion concentrations, although at a certain concentration, ions compete for available water molecules (solvation) and cause polymer precipitation from solutions [106]. NaCMC is an anionic cellulose derivative and it is able to remain in solution in the presence of monovalent cations. On the contrary, divalent cations render NaCMC solutions opalescent and trivalent cations cause polymer precipitation. NaCMC solutions are stable between pH 7 and 9. For pH values below 4, the insoluble form of NaCMC predominates over the water-soluble one [106].

Mechanism of Mucoadhesion Cellulose ethers (HPMC, NaCMC, HPC, HEC) are characterized by good mucoadhesive properties [107, 108].

Especially for the non-ionic derivatives, the mucoadhesive performance is due to the interpenetration of polymer chains and mucin molecules and on the formation of hydrogen bonds [109]. In comparison to anionic polymers, the mucoadhesive

properties of HPC and HEC are not affected by electrolytes [109]. Precipitated HPC was characterized by long-lasting (15-h) adhesion to freshly excised porcine small intestinal mucosa [109]. The adhesion time of the lyophilised (dry) form was in both cases exactly 1.4 times shorter than that of the precipitated polymer, thus indicating that the lyophilisation process reduces the mucoadhesive potential of non-ionic polymers. This could be explained by a higher rate of swelling that may lead to a loss of gel cohesive properties and to a weak mucoadhesive joint [110]. The increase in contact time between the biological substrate and the cellulose derivative gel improves the interaction and strengthens the mucoadhesive joint [110].

NaCMC would be expected to behave much the same, being an anionic polymer with numerous carboxyl acid groups (and hydroxyl groups). However, the gel strength of both polymer and polymer–mucus mixtures remained unaffected by pH [111, 112]. In particular, the viscoelastic properties of both CMC solutions and CMC–mucin mixtures were almost constant over a wide pH range. A possible explanation could be that CMC exists in a coiled conformation due to internal hydrogen bonding at lower pH, and that this conformation remains despite changes in ionisation due to an increased pH. This result may be related to a low charge density, which limits the creation of an expanded polymer network. However, NaCMC may show strong mucoadhesive strength in dry or partially hydrated state when brought in contact with a slightly wet or humid piece of mucosal tissue. This type of mucoadhesion may vanish spontaneously, when the polymer is overhydrated by an excess water amount [37, 112]. Furthermore, in a partially hydrated or dry state, gels based on NaCMC are characterized by better mucoadhesive performance than those of HEC [109]: These results could be explained by the ionic interaction, responsible for the increase in mucoadhesive potential of ionisable polymers [113].

Pharmaceutical Applications in Oral Mucosal Drug Delivery Controlled-release mucoadhesive tablets for gingival/periodontal application were prepared using HPMC and Carbopol (weight ratios 1:2, 1:1 and 2:1) and were loaded with eugenol (10 mg). Incorporation of eugenol in a mucoadhesive formulation provided controlled release for a period of 8 h, which is advantageous over conventional formulation. In vitro mucoadhesion was related to HPMC content of the formulation and correlated well with in vivo performance [114].

HPC matrixes prepared by means of a hot-melt cast moulding method were loaded with Δ9-tetrahydrocannabinol (THC). The incorporation of THC led to an increase in the bioadhesive strength of the HPC polymer matrixes. HPC proved to be a suitable polymer for the development of a mucoadhesive transmucosal matrix system containing THC [115].

4.5.1.5 Polyacrylic Acid-Based Polymers

Carbomers These are high MW carboxyvinyl polymers (Fig. 4.7). Various grades of carbomers are commercially available: they differ with respect to MW, structure and the use of either allylsucrose or allylethers of pentaerythritol as cross-linking

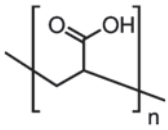


Fig. 4.7 Chemical structure of acrylic acid

agents. Carbomers contain not less than 56% and no more than 68% of carboxylic acid groups, calculated on the dry substance [116].

Carbomer monographs are present in EP and in *United States Pharmacopeia* (USP). While EP contains a single monograph for carbomer, USP presents various monographs relevant to different grades of the polymer (934, 934P, 940, 941, 1342).

Carbomers swell completely only after neutralization with a water-soluble base, with a dramatic increase in viscosity. Such a behaviour is due to dissociation of carboxyl groups in an alkaline environment; the electrostatic repulsion between the negatively charged carboxyl groups causes molecule to uncoil and expand which result in polymer swelling and gel formation [116].

Mechanism of Mucoadhesion It is generally recognized that the presence of polymer un-ionised carboxyl groups plays a relevant role in the formation of the mucoadhesive joint. Physical chain entanglements and hydrogen bonds between carbomer and sialic acid residues of mucin result in the formation of a strong mucus gel network, capable of resisting deformation and allowing the adhesion of the mucoadhesive system for an extended period of time. Some authors found that the addition at pH 6.2 of urea and potassium thiocyanate, two hydrogen-bond-breaking agents, to a mixture of homogenized mucus/Carbopol 934 (MG/C934) resulted in the reduction of the mixture viscoelastic properties, which indicates the reduction of polymer mucoadhesion potential [117]. The mucoadhesive properties of carbomers are widely affected by the environmental pH. An optimum pH of 5.1 was found to produce the strongest gel network of MG/C934 mixtures.

As already mentioned, the formation of the mucoadhesive joint is the result of an interpenetration of polymer and glycoprotein chains, followed by the establishment of secondary (hydrogen) chemical bonds. The interpenetration phenomenon is influenced by the swelling degree of the polymer, which, in turn, is affected by the environmental pH. At alkaline pH, for example, carboxylic groups are expected to be in ionised form, to repulse each other and to give rise to an expanded polymer network available for interpenetration. On the other hand, over-hydration should be avoided, since it could produce a number of polymer chains insufficient to interact with mucin glycoproteins.

While an alkaline pH is optimal for interpenetration, the presence of unionised carboxylic groups (at acidic pH values) is functional for the formation of hydrogen bonds between polymer chains and mucins.

Cross-link density also plays an important role in the mucoadhesion phenomenon. Some authors found that mucoadhesive force increases on increasing cross-link density [118]. According to the authors, this is explained by the increased likelihood of entanglement between polymer and mucin chains, due to an increase in the concentration of polyacrylic acid/unit surface area. Moreover, it is known that

cross-linking diminishes the dissolution rate of hydrophilic polymer chains in an aqueous environment, providing comparatively greater cohesion [109]. However, if cross-link density increases too much, a decrease in polymer mobility occurs, coupled with a reduced opening of the polymer network, which might produce a reduced polymer–mucin interaction.

In conclusion, to realize mucoadhesion, the functional groups responsible for hydrogen bonds must be above a critical concentration (80% for vinyl polymers), and the polymer chains should be flexible enough to form as many hydrogen bonds as possible [119].

Carbomers are highly sensitive to the ionic environment. Their use in an ion-rich environment may interfere with the polymer mucoadhesive performance. Both the viscosity and the viscoelastic moduli of C934P aqueous solutions depend on the hydration medium employed, since it is capable of influencing the polymer network [111]. In the in vitro assessment of carbomer mucoadhesive performance, attention should be given to the choice of the biological substrate. In fact, different mucin types could produce a dramatic difference in the results. No interaction occurred between C934 and two commercial porcine gastric mucins; this result was explained by the presence of ions in the mucin samples (probably derived from the mucin extraction and purification processes), which were responsible for a breakdown of the polymeric network.

The mucoadhesive properties of different carbomers were investigated using porcine small intestinal mucosa as biological substrate [109]. The pH of polymer solutions was found to be a factor influencing the mucoadhesive performance of polymers. Among polyacrylates, Carbopol 980, characterized by the highest MW and by a high cross-linking degree, was found to adhere to the mucosa for the longest time. On the other hand, poly(acrylic acid), with the smallest MW and not cross-linked, showed the shortest time of adhesion.

Carbomers have been widely used in combination with other polymers, particularly celluloses (HPC, HPMC, ethyl cellulose). Being polyanionic, carbomers may complex with cationic and also non-ionic excipients or drugs, thereby modifying their mucoadhesive potential [116, 120].

For example, the addition of polyvinylpyrrolidone (PVP), a well-known mucoadhesive polymer, to C934P was found to reduce significantly the mucoadhesive properties of carbomer [121]. Since PVP presents an electron-deficient region, it acts as a hydrogen bond acceptor; this determines an interaction between PVP and carboxylic acid groups of carbomer, to the detriment of the carbomer–mucin interaction.

Another mechanism was proposed by Mortazavi and Smart [122] to explain the mucoadhesive properties of carbomers. The authors observed that the water movement could play an important role in mucoadhesion when dry or partially hydrated dosage forms are involved. Carbomers were found to dehydrate the mucus more than the neutral polymers. This produced the increase in adhesive and cohesive structure of the mucous gels and the strengthening of the weakest components of a mucoadhesive joint.

Pharmaceutical Applications in Oral Mucosal Drug Delivery Carbomers have extensively been used in the formulation of mucoadhesive controlled drug delivery systems.

In particular, carbomers have been employed to develop drug delivery systems intended for transmucosal and mucosal administration of drugs via different routes such as ophthalmic, nasal, buccal and vaginal.

As for buccal administration of drugs, films based on polyacrylic acid sodium salt (Carbopol EX 214) and chitosan mixtures were developed for buccal delivery of acyclovir. Optimal mucoadhesion was obtained with a certain weight ratio between the two polymers. The formation of an inter-polymer interaction product allowed the modulation of film hydration, which, in turn, affected mucoadhesive and drug release properties of the formulation [61].

Poly(acrylic) Acid and Poly(acrylic acid) Derivative Copolymers Several poly(acrylic acid) copolymers have been shown to possess good mucoadhesive properties toward different mucosae.

In particular, mucoadhesive systems based on a copolymer of acrylic acid (AA) and poly(ethylene) glycol were shown to be good candidates for controlled oral mucosal delivery of acyclovir [123].

In other work, hydrogels based on poly(acrylic acid-g-ethylene glycol) (P(AA-g-EG)) copolymers were synthesized using two PEG derivatives having different MWs and with different molar ratios of AA–EG [124]. The effects of different PEG-tethered structures on mucoadhesion were studied. Preswollen P(AA-g-EG) copolymer films composed of 40 % AA and 60 % ethylene glycol (EG), containing PEG 1000 tethers, exhibited the highest work of mucoadhesion, five times higher than the formulation based on pure poly(acrylic acid). The authors concluded that the higher mucoadhesive properties of this copolymer were due to the synergistic effects of both monomers. AA functional groups allowed the polymer to form multiple hydrogen bonds with the glycoproteins present in the mucus. PEG tethers possibly acted as mucoadhesive promoters, enhancing the interpenetration of polymer chains into the mucus.

Shojaei et al. designed copolymers of AA and 2-ethylhexyl acrylate (EHA), P(AA-co-EHA), for buccal mucoadhesion. A series of linear copolymers with different molar ratios of the two monomers were synthesized [125]. The copolymer made up of 46:54 mol.% AA:EHA yielded the highest mucoadhesive force in contact with porcine buccal mucosa, significantly greater than that of PAA (used as positive control).

A series of novel mucoadhesive poly(acrylic acid-co-poly(ethylene glycol) monomethylether monomethacrylate-co-dimethylaminoethyl methacrylate) (poly(AA–PEGMM–DMEMA)) were synthesized by incorporating the cationic monomer DMEMA into poly(AA–PEGMM) to enhance the interactions between the mucoadhesive polymer and the buccal mucosa [126]. An attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) study revealed that intra-polymer interactions and inter-surface interactions played opposite roles in the mucoadhesion performance of the polymers. Optimal mucoadhesion can be achieved by balancing these two interactions.

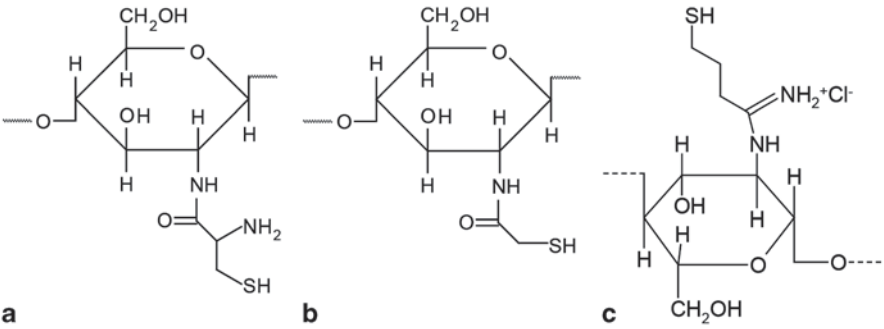


Fig. 4.8 Chemical structure of chitosan–cysteine (**a**) chitosan–thioglycolic acid (**b**) and chitosan–thiobutylamidine (**c**)

4.5.2 Second-Generation Mucoadhesive Polymers Used in the Oral Cavity

Unlike first-generation non-specific platforms, certain second-generation polymer platforms are less susceptible to mucus turnover. Furthermore, as surface carbohydrate and protein composition at potential target sites vary regionally, more accurate drug delivery may be achievable [127].

Thiolated polymers, lectins and bacterial adhesions are classified as second-generation mucoadhesive polymers. Thiolated polymers (thiomers) derive from hydrophilic polymers such as chitosan, polyacrylates or deacetylated gellan gum [128], alginate–cysteine, poly(methacrylic acid)–cysteine and sodium carboxymethyl cellulose–cysteine [127]. The mucoadhesive properties of these polymers are sensibly improved in comparison with the parent moiety: for instance, the increase was at least 140-fold for chitosans [129] and 20-fold for polyacrylates [130].

4.5.2.1 Thiolated Mucoadhesive Polymers

Thiolated Chitosans These are cationic polymers chitosan–cysteine (Fig. 4.8a), chitosan–thioglycolic acid (Fig. 4.8b) and chitosan–thiobutylamidine (Fig. 4.8c) [131]. The thiolated chitosans are cationic polymers in which the primary amino group in the second position of the glucosamine chitosan subunits is substituted with residues presenting a thiolic group. Sulphydryl-bearing agents can be covalently attached to this primary amino group via the formation of amide or amidine bonds.

In contrast to well-established mucoadhesive polymers, these thiolated chitosans are capable of forming covalent bonds. The bridging structure most commonly encountered in biological systems (the disulphide bond) has thereby been discovered for the covalent adhesion of polymers to the mucus gel layer of the mucosa. Based on thiol/disulphide exchange reactions [132] and/or a simple oxidation process

[133], disulphide bonds are formed between such polymers and cysteine-rich subdomains of mucus glycoproteins [128, 131]. Hence, thiomers mimic the natural mechanism of secreted mucus glycoproteins, which are also covalently anchored to the mucus layer by the formation of disulphide bonds.

The mucoadhesive properties are also due to the formation of disulphide bonds within the thiomer itself after the interpenetration process, leading to additional anchors via chaining up with the mucus gel layer. These covalent bonds between thiolated chitosan and mucus or the cross-links are supposedly stronger than non-covalent bonds, such as ionic interactions of chitosan with anionic substructures of the mucus layer.

With chitosan–thioglycolic acid conjugates a five- to tenfold increase in mucoadhesion in comparison to unmodified chitosan was achieved. The mucoadhesive properties of chitosan–thiobutylamidine conjugates were even further improved up to 100-fold and 140-fold with respect to the starting chitosan. One explanation for this phenomenon can be given by the theory that chitosan–thiobutylamidine conjugates possess increased mucoadhesive properties due to improved ionic interactions between the additional cationic amidine substructure of the thiomer and anionic substructures within the mucus layer. The MW influences the mucoadhesive properties: medium MW polymers exhibited better mucoadhesive performance.

Thiolated chitosans caused increased drug absorption due to opening of tight junctions. This effect was improved by the addition of the permeation mediator glutathione (GSH) [20, 134, 135]. The likely mechanism of action of thiomer/GSH systems has been ascribed to the capability of GSH to inhibit the enzyme protein tyrosine phosphatase (PTP). Such an enzyme is able to dephosphorylate tyrosine residues of occludin. This dephosphorylation results in the closing of tight junctions. The inhibitory effect of GSH is limited as it is rapidly oxidised on the cell surface. Thiomers are capable of reducing oxidised glutathione to GSH. A high concentration of GSH on the membrane should result in an opening of tight junctions.

Thiolated Poly(acrylates) These are anionic polymers such as (poly)carbophil–cysteine [136], poly(acrylic acid)–cysteine, poly(acrylic acid)–cysteamine and poly(acrylic acid)–homocysteine (Fig. 4.9) [131]. Other thiolated polyacrylates are also available. These polymers were shown to be stable when stored in the dry form. In fact, they form disulphide bonds in a pH-dependent manner in aqueous solution.

Some authors demonstrated that such polymers could be stabilised in aqueous solution when the liquid formulations are produced under inert conditions and particular storage precautions are taken (vessels in aluminium foil containing an oxygen scavenger). As previously discussed, the formation of disulphide bonds between the thiomers and the mucus, either via thiol/disulphide exchange reaction or via a simple oxidation process of free thiol groups, is responsible for the mucoadhesive joint.

Another likely mechanism responsible for the mucoadhesive properties of such polymers is based on their in situ cross-linking properties: during and after the interpenetration process, disulphide bonds are formed within the polymer leading to additional anchors, thanks to chaining up with the mucus gel layer. Thiolated poly(acrylates) were proved to possess increased mucoadhesion properties.

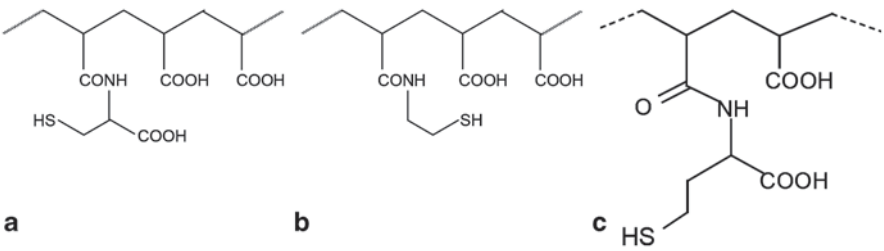


Fig. 4.9 Chemical structure of poly(acrylic acid)–cysteine (**a**), poly(acrylic acid)–cysteamine (**b**) and poly(acrylic acid)–homocysteine (**c**)

Oral delivery systems containing low molecular weight heparin (LMWH) and insulin were developed [137, 138]. Thiolated poly(acrylic acid)-based formulations (gel, microspheres) were successfully used for nasal delivery of human growth hormone (hGH) [139]. Eye drops intended for the treatment of dry eye syndrome and ocular inserts based on thiolated poly(acrylic acid) were developed [131, 140, 141].

4.5.2.2 Lectins and Bacterial Adhesions

Lectins and bacterial fimbrins and invasions more accurately termed "cytoadhesives" are second-generation polymer platforms less susceptible to mucus turnover rates, since they can bind directly to mucosal surfaces. Lectins are naturally occurring proteins that play a fundamental role in biological recognition phenomena involving cells and proteins. After initial mucosal cell binding, lectins can either remain on the cell surface or be internalized via endocytosis in the case of receptor-mediated adhesion [142]. Although lectins offer significant advantages in relation to site targeting, many are toxic or immunogenic, and the effects of repeated lectin exposure are largely unknown. It is also feasible that lectin-induced antibodies could block subsequent adhesive interactions between mucosal epithelial cell surfaces and lectin delivery vehicles. Moreover, such antibodies may also render individuals susceptible to systemic anaphylaxis on subsequent exposure [142].

4.6 Methods to Assess Mucoadhesive Properties

Various mechanical testing methods have been used to assess the adhesive properties of mucoadhesive materials and formulations depending on the polymers and the drug delivery systems considered. The most common tests used in the literature are: physical tests and in particular rheological ones, mechanical tests and in particular measurements of tensile forces and dynamic indirect tests, among which the more relevant ones are washability test and inclined plane test.

4.6.1 Rheological Tests

The rheological approach is commonly used to evaluate mucoadhesion; this technique investigates the changes in the rheological properties of the mucoadhesive polymer when mixed with mucus or mucins. In particular, the method is based on the concept that the mixture of a mucoadhesive polymer and mucin is characterized by a synergic increase in viscosity with respect to the viscosity of each component, either polymer solution or mucin dispersion. This phenomenon is due to chain interlocking, conformational changes and chemical interactions between polymer and mucin chains [18, 143, 144].

As initially proposed by Hassan and Gallo [143], rheological synergism may be simply calculated using apparent viscosity values measured at a prefixed shear rate as follows:

$$\Delta\eta = \eta(\text{mixture}) - (\eta(\text{mucin}) + \eta(\text{polymeric solution})),$$

where

η(mixture) Apparent viscosity at prefixed shear rate of the polymer-mucin mixture
η(mucin) Apparent viscosity at prefixed shear rate of the mucin dispersion
η(polymeric solution) Apparent viscosity at prefixed shear rate of polymeric solution.

The rheological synergism is directly related to polymer–mucin interaction and consequently describes the mucoadhesive potential of the polymers. However, this method is suited to test Newtonian solutions or weak gels (relatively low G′ values), whereas its significance is questionable for testing strong gels [145].

Both freshly prepared and commercial mucins can be employed as biological substrates and present advantages and drawbacks. Fresh mucins, derived from a small number of animals, are always characterized by a high variability and can give highly variable results. On the contrary, commercial mucins show lower batch variability and are ready to use and can furnish more reliable results but could present crucial differences with respect to the native samples due to the preservation processes that can change mucin structure [111].

Rheological synergism is strongly dependent on the polymer (chemical structure, branched or linear conformation, MW) and also on polymer concentration and viscosity, mucin type and concentration and environmental conditions (pH and ionic strength). Given these considerations, this method may be used to score the mucoadhesive potential of polymers having similar chemical nature (for example, a series of chitosans or a panel of chitosan derivatives) [145].

4.6.2 Mechanical Tests (Tensile Test)

Among the various mechanical approaches reported in the literature to measure mucoadhesion, the most common one is based on the measurements of tensile forces

[61, 64, 146]. This approach allows the evaluation of the strength of the interfacial layer formed between polymer and mucus/mucin. In particular, the force required to detach the sample from a biological substrate as a function of the displacement occurring at the mucoadhesive interface is the key parameter measured. Despite a large number of home-made systems, currently, the most employed apparatus is a texture analyser (TAXT plus Texture analyser, Stable Micro Systems) equipped with the measuring system A/MUC. In this arrangement, the biological substrate (mucin or mucus) is fixed to the A/MUC measuring system (eventually thermostated to a specific physiological temperature (37 or 32 °C), depending on the application/administration site) and the polymeric material is applied to the cylinder probe facing the biological substrate. Polymer sample and biological substrate are put in contact by lowering the cylinder probe, allowing the formation of mucin/polymer interface, and the force needed to detach the sample and mucin is recorded as a function of displacement at the mucoadhesive interface.

This test can be performed by using different biological substrates other than mucus and mucin, in particular mucosal tissues. The employment of mucosal tissues presents a critical issue related to reproducibility. To reduce result variability, the employment of commercial mucin is recommended. Moreover, some instrumental parameters (preload, contact time and detachment rate) play a key role in the reliability of the results and must be carefully chosen and fixed [145]. The tensile test can be profitably employed to study the mucoadhesive performance of solid dosage forms such as tablets or films or of semisolid preparations such as hydrogels or gels.

As for solid systems, since the hydration is one of the critical steps involved in mucoadhesion, hydration volume and time and pH and ionic strength of the medium are critical points that can influence system mucoadhesive performance. Sustained preload and cyclic stress testing have also been reported in the literature to evaluate the durability of the bioadhesive joint [146].

Maximum force of detachment (directly measured) and the work of adhesion (AUC; calculated as the area under the force vs. displacement curve) are the parameters used to evaluate the mucoadhesive potential. However, when it is necessary to compare samples with different cohesive properties, normalization of parameters is required: for this purpose, the tensile measurements are performed by using hydrated filter paper instead of a biological substrate to evaluate the cohesive force while the mucoadhesion at the mucosal interface is evaluated using a mucin layer.

The reliability of the tensile method is strictly dependent on the failure in the interfacial (mucin/polymer) region: in particular, it is difficult to distinguish where the failure of the mucoadhesive joint occurs and if the cohesive nature of the sample (failure within the polymer layer) or the strength of the mucus layer (failure within the mucus layer) plays the major role.

4.6.3 Inclined Plane Test

Among dynamic indirect tests, the one based on inclined plane gives information about the permanence of the mucoadhesive polymeric material in contact with the

biological substrate (mucin film or mucosal tissue). The inclined plane apparatus basically consists of a Plexiglas support (surface area = 28 cm^2) with an angle of inclination between 30° and 60°, thermostated at 37 °C and placed above an electronic microbalance interfaced with a personal computer. The Plexiglas support is coated with a mucin film (prepared by casting) or covered with mucosal tissue. The polymeric material is placed onto the mucin film on the top of the Plexiglas support, still held in the horizontal position; then the plane is inclined and the amount of formulation dropped on the microbalance is recorded as a function of time.

Inclination angle can influence test results and their reliability and should be optimized to reduce sample variability and to better discriminate sample performance. This approach could also be employed to test mucoadhesive systems characterized by in situ gelling properties, since it enables to evaluate the contribution of gelation time to mucoadhesive performance [147].

4.6.4 Washability Test

The washability test is a dynamic indirect test which allows the investigator to mimic the various events that influence the permanence of a formulation following in vivo application on a mucosal tissue. In particular, the retention/permanence of a drug or of a labelling molecule loaded in the mucoadhesive system can be evaluated upon contact with the biological substrate over which a physiological fluid (saliva, vaginal secretions, tears) is fluxed to simulate its removal action.

The apparatus consists of a Franz diffusion cell with a modified donor chamber as described in Bonferoni et al. [148] and Rossi et al. [149]. Briefly, the donor chamber, closed in the upper part, has two side arms which allow a buffer stream over the sample to simulate the washing action of biological fluids. Drug washed away from the biological substrate is collected in a beaker and quantified using a suitable analytical method. This method allows the capability of a drug delivery system to maintain the contact between the loaded drug and the biological substrate to be assessed.

4.6.5 Final Remarks

Although only a few buccal drug delivery systems based on mucoadhesive polymers are present on the market, the oral cavity still remains an advantageous route of administration with the possibility to increase the residence time of the polymer and to achieve site-specific adhesion. The mechanism by which a mucoadhesive joint is formed is strictly related to the functional properties of the mucoadhesive polymer and also depends on the hydrated state of the formulation. Furthermore, the mucosa of the oral cavity and the environment play an important role on mucoadhesive bond formation and on the subsequent joint consolidation.

Multifunctional polymers characterized not only by mucoadhesive properties but also by penetration enhancement and enzymatic inhibition properties should have a central role as enabling excipients for the buccal delivery of a wide variety of therapeutic compounds and in particular of therapeutic macromolecules. In fact, these features are fundamental for the delivery of biotechnological drugs, especially peptides and proteins, which are often characterized by poor bioavailability due to high MW, marked hydrophilicity and sensitivity towards enzyme degradation.

The experimental approaches used to investigate mucoadhesion are crucial to determining the mucoadhesive potential of a drug delivery system and could be fundamental in the pharmaceutical development of a product. To properly evaluate the mucoadhesive potential, the use of integrated techniques is necessary to evaluate the different mucoadhesion mechanisms which are peculiar to different polymeric materials or different formulations.

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Chapter 5
Physical Methods for Enhancing Oral Mucosal Delivery: Sonophoresis, Iontophoresis and Electroporation

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5.1 Introduction

Currently, the interest in oral mucosal permeability is related to the opportunity of using the tissue for controlled drug delivery for both local and systemic purposes. It is also assumed that permeability of mucosa might be involved in the aetiology of certain oral mucosal diseases, including premalignant conditions and cancer.

The lining mucosa of the oral cavity includes three different types of mucosal tissues that vary considerably in structure, thickness and permeability along the different oral regions. Among these mucosal tissues, the buccal and sublingual mucosae offer an easily accessible and generally well-accepted site for delivering systemically acting drugs.

The passage of drugs through the oral mucosa is a complex event and reflects the structure of the tissue, the pathologic status as well as the nature of the penetrants [1–3].

For systemically acting drugs, mostly for the treatment of chronic diseases, the rate at which molecules cross the oral mucosa determines the therapeutic effect profile (e.g. onset, intensity and duration of action) of the active. The success of drug delivery through oral mucosa depends on the ability of the drug to permeate the mucosal barrier at a concentration high enough to achieve its desired therapeutic effect.

Drugs can be transported across epithelial membranes by passive diffusion, carrier-mediated active transport or other specialized mechanisms. The most important mechanism of drug passage is spontaneous transfer of a solute that moves from a point of higher chemical potential to a point of lower chemical potential. This phenomenon (passive diffusion) can be described by the general diffusion equations that, however, do not take into account the complex arrangement of the mucus network in the oral cavity. Mucus forms an adherent, unstirred, viscoelastic layer

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adjacent to the epithelial surface and acts as a robust barrier that could constitute the major obstacle to drug transport [4].

Among the various mucosae of the oral cavity, the buccal and sublingual mucosae are the most absorptive. They possess hydrophilic pores that are formed by the hydrophilic headgroups of lipids, and are readily filled with water molecules, therefore forming lipid bilayers. On the other hand, the bilayers give rise also to hydrophobic domains formed by the hydrophobic lipid tails that slow down the passage of hydrophilic materials across the epithelium [5, 6]. Drugs diffuse through epithelial cells into the submucosa via the intracellular (transcellular; through cells) or via the intercellular (paracellular; where material passes through aqueous, lipid-rich domains around the cells) pathways, along a chemical potential gradient. The gradient slope depends on physico-chemical properties of the drug (e.g. solubility, partitioning, stability, physical state, thermodynamic activity, molecular size, and pK_a), thickness of the barrier and rate of blood perfusion in the submucosa. The diffusion rate is both compound specific and region selective.

Absorption of lipophilic compounds is generally considered to occur through transcellular diffusion. Hydrophilic compounds appear to be absorbed via paracellular diffusion through intercellular spaces. In the most part, drugs can diffuse using both routes simultaneously, although the route with the least penetration resistance is usually preferred over the other.

Similar to other mucosal membranes, the buccal mucosa has some limitations including short residence time, small absorption area, and low permeation rate of therapeutic agents. The barrier properties and the diffusion through epithelial tissues could be mitigated by the use of suitable mucoadhesive formulations, permeability enhancers, enzyme inhibitors and vehicle/cosolvents as well as drug modifications (e.g. prodrugs) [5, 7–11]. Nevertheless, several studies have reported drawbacks for each of these methods. It has been demonstrated that synthetic mucoadhesive nanoparticles can extensively alter the microstructure of mucus, causing disruption of the mucus barriers that, in turn, implies exposure to foreign particles, including pathogens and other potentially toxic nanomaterials [12].

Chemical penetration enhancers have been widely studied to improve the delivery of diffusants across the buccal mucosa. Although many chemicals have been evaluated as penetration enhancers in human or animal models, to date none has proven to be ideal. It is difficult to rationally select a penetration enhancer for a given permeant. The efficacy of penetration enhancers appears to be drug specific and depends on physico-chemical properties of the drug; at best, it may be predictive for a series of permeants with similar properties (such as similar distribution coefficients, molecular weights and solubility) [13–16].

Various substances have been explored as efficient permeation enhancers to increase the flux/absorption of drugs through the mucosa; however, irritation, membrane damage and toxicity are often associated with them and could limit their use [16].

Hence, alternative and/or concurrent methods of enhancing permeation, which are safe as well as effective, have been investigated. Among them, physical means (e.g., sonophoresis, iontophoresis and electroporation) could be employed or developed for drug delivery in the oral cavity, thereby expanding the current drug candidate list for this area [17].

5.2 Sonophoresis

To assist drug permeation, many delivery techniques that use different forms of energy have been explored. Ultrasounds at various frequencies in the range of 20 kHz to 16 MHz with intensities of up to 3 W/cm^2 have been used to promote drug transport (sonophoresis) [18, 19]. Usually, the area of its application is transdermal drug delivery, but it may also be used for enhancement of drug transfer through mucosal tissues.

After topical application of actives, sonophoresis exponentially increases absorption of compounds into the tissues. Numerous studies have shown that this technique, applied to transdermal delivery, offers promising potential for a local, non-invasive, convenient and rapid method to promote permeation of low molecular weight drugs as well as macromolecules [20].

Mechanistically, sonophoresis is considered to enhance drug delivery through a combination of thermal, chemical and mechanical alterations within the tissue [21]. Drug absorption occurs because ultrasound waves stimulate micro-vibrations within the epithelium and increase the overall kinetic energy of permeants. However, extensive literature on ultrasound drug delivery is not confined only to transdermal applications but also covers other tissues (e.g. sclera) and gene delivery [22].

Sonophoresis has the advantage of improved drug penetration over passive transport, allowing strict control of the transepithelial penetration rate. Control of drug plasma levels is feasible, especially for actives characterized by a small therapeutic index; drug doses and dosing frequency are reduced and patient compliance is increased. Since the tissue remains intact, low risk of infection occurs [23–25]. Nevertheless, some disadvantages may also occur, among which are: The technique is functional just if applied on intact tissues, there is minor irritation, burning and tingling could arise and the method is time consuming [26, 27]. Ultrasound is typically classified based on frequency: high-frequency or diagnostic ultrasound (above 3 MHz), medium-frequency or therapeutic ultrasound (f ~1–3 MHz) and low-frequency or power ultrasound (20 kHz$<f<$100 kHz).

To enhance skin permeability, different ultrasound frequencies have been used; however, it has been found that drug transport enhancement induced by low frequencies ($f<$100 kHz) is more significant than that induced by high frequencies. It is remarkable that low-frequency ultrasound enhances transdermal drug transport 1000 times more than high-frequency ultrasound [21, 28–30].

Over the years, low-frequency ultrasound has been practised using two different types of application: simultaneous sonophoresis and pretreatment sonication. Simultaneous sonophoresis corresponds to a concurrent application of drug and ultrasound to the epithelium. This method enhances tissue transport mainly attributable to structural alterations of the epithelium induced by ultrasound. This type of sonophoresis requires that the patient use a wearable ultrasound device for drug delivery.

In pretreatment sonication, a short application of ultrasound is used to permeabilize the tissue prior to drug delivery. The tissue remains in a state of high permeability for several hours during which drugs can be delivered. In this approach, the patient does not need to wear any device [31].

5.2.1 Principles of Sonophoresis

Acoustic waves with frequencies between 20 Hz and ~20 KHz fall in the audible range. The term "ultrasonic" refers to sound waves whose frequency is >20 KHz [32]. There is a direct relationship between the wave rate, frequency and wavelength. The intensity is progressively lost when a sound wave passes through the body or is deviated from its initial direction. This phenomenon, referred to as "attenuation", in homogeneous tissues occurs as a result of absorption; in this case, the sound energy is transformed into heat and scattering [23].

As the frequency increases, the vibration amplitude falls, attenuation increases, and all the energy is dissipated over a short distance. Accordingly, the wavelength of ultrasound plays a significant role in drug delivery. The resistance of the medium to the propagation of sound waves is dependent on the acoustic impedance (Z) which, in turn, is related to the mass density of the medium (ρ) and the speed of propagation (C), according to:

$$Z = \rho \times C \tag{5.1}$$

The specific acoustic impedances for skin, bone and air are 1.6×10^6, 6.3×10^6 and 400 kg/(m^2s), respectively. As ultrasound energy penetrates the body tissues, biological effects can be expected to occur when energy is absorbed. The absorption coefficient is used as a measure of this phenomenon. For ultrasound consisting of longitudinal waves with perpendicular incidence on homogeneous tissues, the following equation is applied:

$$I_{(x)} = I_0 \times e^{-ax}, \tag{5.2}$$

where

$I_{(x)}$ Is the intensity at depth x
I_0 Is the intensity at the surface
a Is the absorption coefficient

Because of the high impedance of air, to transfer ultrasound energy to the body, the use of a contact medium is necessary. Most types of contact media currently available for ultrasound transmission can be broadly classified as oils, water–oil emulsions, aqueous gels and ointments [33].

5.2.2 Dependence of Transport on Ultrasound Parameters

The extent of enhancement induced by low-frequency sonophoresis depends on four main acoustic parameters: frequency, intensity, duty cycle and application time of ultrasound.

Frequency Low-frequency ultrasounds ($f \sim 20$ kHz) are significantly more effective in enhancing tissue permeability compared to therapeutic ultrasounds. A detailed investigation of the dependence of permeability enhancement on frequency and intensity in the low-frequency regimen (20 kHz$<f<$100 kHz) has been reported [29]. Although low frequencies induce high enhancement, the transport at low frequencies was found to be localized to certain areas, the so-called localized transport pathways [29]. With an increase in ultrasound frequency, the transport was found to be more homogeneous and the optimum appears to be around 60 kHz. Close to this value, significant transport enhancement can be obtained with reasonable energy doses together with sufficient homogeneity of the transport pathways. Attenuation of acoustic waves is inversely proportional to the frequency; as the frequency increases, ultrasounds penetrate less deeply into the tissues.

Intensity The tissue conductivity increases with the increase of intensity up to a certain level and then drops off, most likely due to an increase of the total energy delivered to the system. The linearity of the conductivity/ultrasound intensity ratio may fail at higher intensities ($I>15$ W/cm^2) due to other effects such as "acoustic decoupling". This phenomenon is generated near the ultrasound source and results in the formation of large number of gaseous cavities; thus, the amount of energy delivered to the system is reduced.

At each frequency exists an intensity below which enhancement is not detectable; this intensity is referred to the threshold intensity. Once the intensity exceeds this threshold, the enhancement increases strongly with the intensity (e.g. the threshold intensity for porcine skin increases from about 0.11 W/cm^2 at 19.6 kHz to more than 2 W/cm^2 at 93.4 kHz). The origin of this extensive increase in the threshold intensity with frequency may be attributed to the phenomenon of cavitation (see Sect. 5.2.3.), which plays a major role in low-frequency sonophoresis. Usually, the employed intensity ranges from 0.5 to 2 W/cm^2 [26].

Intensity can be combined with duty cycle and application time into a single parameter, the total energy density delivered from the transducer (E_d):

$$E_d = \text{intensity} \times \text{exposure time} \times \text{duty cycle}. \tag{5.3}$$

In other words, the effect of ultrasounds on tissue permeability is comparable if the total energy density delivered to the tissue is maintained constant. To have an effect on skin permeability, the threshold energy density should be about 222 J/cm^2 [34]. The magnitude of the threshold depends on the type of tissue and may vary between different tissue models.

5.2.3 Mechanisms of Action and Physical Effects of Ultrasound Waves

Although sonophoresis has been extensively studied over the years, all mechanisms implied are not clearly understood reflecting the fact that several concurrent phenomena may occur in the biological tissue upon ultrasound exposure. Among them,

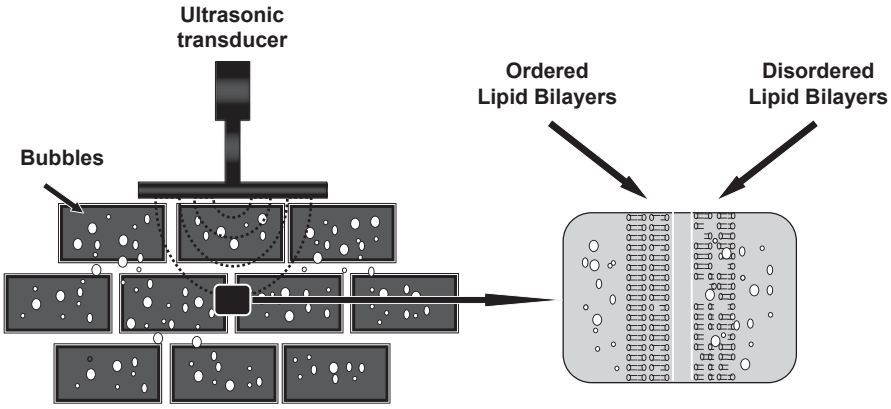

Fig. 5.1 Schematic sketch of cavitation occurring in epithelial tissues following low-frequency ultrasound delivery

cavitation effects, thermal effects, induction of convective transport and mechanical effects are the most significant.

Cavitation Effects Exposure to ultrasounds gives rise to "cavitation" which refers to the formation of gaseous cavities (bubbles) primarily caused by the ultrasound-induced variation of pressure in the treated medium [23].

Acoustic-generated cavitation bubbles interact with biological tissues, playing an important role in biomedical applications, given that the bubbles microscopically disrupt the lipid bilayers of membranes. Low-frequency ultrasounds are able to generate microbubbles in both water and tissue.

Cavitation occurs in a variety of mammalian tissues including muscle, brain and liver, upon exposure to ultrasound in different conditions. This occurrence is attributed to the existence of a large number of gas pockets trapped in either intracellular or intercellular structures. The effects vary inversely with ultrasound frequency and directly with ultrasound intensity [23, 29, 32, 33].

Cavitation is of two types: the inertial (transient) cavitation which leads to a rapid growth and collapse of bubbles, and the stable cavitation which implies slow oscillatory motion of bubbles in an ultrasound field [36]. It seems that the transient cavitation is primarily responsible for skin permeabilization [37].

Nucleation of small gaseous cavities, caused by negative pressure generated during application of ultrasound, is followed by the growth of cavitation bubbles. Subsequent collapse of cavitation bubbles releases a shock wave that, in the surrounding tissue, can cause structural alteration, which results in the formation of water channels within the lipid bilayers. Whenever small gaseous nuclei already exist in a medium, cavitation takes place preferentially at those nuclei. The lipid bilayers disordering together with the formation of aqueous channels allow permeation of hydrophilic drugs. Cavitation occurs preferentially at the interface between the epithelial cells and the lipid bilayers [23] (Fig. 5.1).

For skin penetration enhancement, three major mechanisms of cavitation-mediated permeabilization have been suggested: (i) disordering of lipid bilayer due to the release of shock waves, (ii) impact of acoustic microjets on tissue surface and (iii) microjet penetration into the tissue [38].

In low-frequency sonophoresis, a strong role is played by inertial cavitation which depends on ultrasound intensity [39, 40]. In particular, exceeding ultrasound threshold intensity is required for inception of inertial cavitation. This threshold represents the minimum pressure amplitude required to induce rapid growth and collapse of gaseous nuclei. The threshold intensity increases with the increase in ultrasound frequency given that the growth of cavitation bubbles is progressively more difficult [40].

Based on a porous pathway model of transdermal transport of hydrophilic solutes, it has been suggested that during low-frequency sonophoresis solute transport is mainly affected by the increased porosity of the tissue [41].

Thermal Effects The thermal effects of ultrasound on the application site are the consequence of the transfer and conversion of mechanical energy generated in the sonophoresis probe. During its propagation, the ultrasound wave is partially scattered and absorbed by the tissue medium resulting in attenuation of the emitted wave. An aliquot of lost energy is converted into heat while the remainder of the wave penetrates into the tissue and propagates through the medium, thus increasing its temperature [33]. Since tissue permeability increases significantly with temperature, it is consequent that thermal changes contribute to sonophoretically enhanced transport.

Every medium absorbs ultrasound to a certain extent, and the aptitude to do it is described by the ultrasound absorption coefficient: Materials that possess high ultrasound absorption coefficients (e.g. bone) experience severe thermal effects when compared to those with low absorption coefficient (e.g. muscle tissues) [42]. The absorption coefficient can give indications about the extent of temperature rise following sonication with a beam of known intensity. Upon ultrasound exposure, the increase in the temperature varies directly with the ultrasound intensity and exposure time. However, only about one fourth of enhancement is attributable to the increase of temperature following application of low-frequency sonophoresis [43].

Convective Transport The application of ultrasound in fluids is known to produce convective flow patterns inside tissues and cells. Due to interference of the incident and reflected ultrasound waves and oscillations of the cavitation bubbles, fluid movements are generated and transport of permeants is promoted. However, experimental findings suggest that convective transport does not play an important role in transdermal enhancement [44].

Mechanical Effects The longitudinal pressure wave created by ultrasound application induces sinusoidal progressive pressure variations in the tissue and, accordingly, sinusoidal density changes. These variations occur very rapidly at frequencies greater than 1 MHz so that gaseous nuclei cannot grow and cavitational effects cease. Sinusoidal density changes could generate recurring stresses, and lipid bilayers can easily be disordered by these stresses, resulting in an increase of permeability. However, cavitation-induced lipid bilayer disordering is the most important cause for ultrasonic enhancement of transepithelial transport [44].

5.2.4 Effects of Sonophoresis on Biological Tissues and Safety

In order to safely apply low-frequency ultrasound, the selection of appropriate parameters is crucial. Several parameters, including frequency, intensity, duty cycle, application time, distance of transducer and tissue type, can affect safety.

Significant efforts have been made to evaluate the safety of low-frequency ultrasound exposure in clinical and laboratory studies [45]. The effects of ultrasound on the integrity of human skin structure have been evaluated in vitro. Skin specimens were exposed to 20-kHz ultrasound with average intensities ranging from 0.25 to 7 W/cm^2 in pulsed or continuous mode. Using optical and electron microscopy, no structural changes or damages have been highlighted in the skin samples or in the underlying tissues exposed to ultrasound [46]. Nevertheless, in hairless rat skin samples, slight and transient erythema was observed after 2.5W/cm^2 exposures and deep lesions were observed 24 h later; these lesions are not attributable to the increase in temperature [46].

The World Federation for Ultrasound in Medicine and Biology (http://www.wfumb.org) has issued several publications relating to the safety of ultrasound bioeffects and nonthermal bioeffects, in an attempt to adopt a policy on safety guidelines [47, 48]. However, further research focusing on the safety issues is required to evaluate the limiting ultrasound parameters.

5.2.5 Prediction of Sonophoretic Enhancement

Although the basic processes of low-frequency ultrasound have been already recognized, it is still difficult to predict the extent of drug delivery enhancement produced at any given application. This is due to the fact that the extent of cavitational disordering depends upon numerous variables (e.g. the distance between the transducer and the tissue, the transducer geometry, the distribution of dispersed bubbles). Also, the properties of permeant may contribute to transport enhancement. However, a qualitative prediction of the sonophoretic enhancement of transepithelial transport can be made with good rightness on the basis of knowledge of physico-chemical properties of permeants. In particular, using the passive skin permeability (P^{P}) and octanol–water partition coefficient ($K_{o/w}$), it is possible to obtain the relative sonophoretic transport enhancement E:

$$E = \left[\frac{\text{sonophoretic permeability}}{\text{passive permeability}} - 1 \right] \cong \frac{K_{o/w}^{\ 0.75}}{(4 \times 10^4) P^{P}}. \tag{5.4}$$

Drugs having a predicted E value smaller than 1 exhibit no sonophoretic enhancement (e.g., lidocaine and salicylic acid) whereas those having a predicted E value equal to 1 or more exhibit sonophoretic enhancement (e.g., hydrocortisone and indomethacin) [49, 50]. Generally, drugs that diffuse through the skin passively and slowly are

enhanced by the application of ultrasound. Low-frequency ultrasound has primary effects on the drug diffusion coefficient rather than on the partition coefficient [51].

5.2.6 Experimental Protocol for Ex Vivo Study of Sonophoresis

Recently, a suitable protocol that could be used to measure ex vivo percutaneous enhancement of drug transport during ultrasound-assisted delivery has been proposed. In this protocol, skin obtained from porcine ears is used as a model barrier. Tissue specimens are mounted in vertical Franz-type diffusion cells using phosphate-buffered saline (PBS) as receptor solution. Low-frequency (20 kHz) ultrasound is then applied for different durations (range: 5 s to 10 min) using distinct sonication procedures either before or concurrent with drug deposition. In pretreatment experiments, sonication is undertaken immediately after hydration of the skin but prior to the topical application of the drug solution, whereas in concurrent studies, sonication is undertaken immediately after topical deposition of the drug solution. Finally, drug flux is measured and enhancing effects determined. Using this protocol and caffeine as a hydrophilic model drug, it was found that sonication concurrent with drug deposition was superior to sonication prior to drug deposition [52].

5.2.7 Methods and Devices Patented for Sonophoresis

A transducer is a device used to transmit and detect ultrasound. In these devices, the electrical energy is converted into sound energy to produce ultrasound waves. Over the last decade, various transducers have been described and patented to attain ultrasound-enhanced drug permeation through dermal or mucosal membranes.

A method using ultrasound for enhancing and controlling drug permeation through the buccal membrane and reach therapeutic levels into the circulatory system is described in US 4948587 patent [53]. The frequency and intensity of ultrasonic energy which is applied, and the exposure time are determined according to the location and nature of the buccal membrane and the substance to be infused [53]. This improved method and a device to be applied to small areas of skin is described in US 6234990 B1 patent [54].

An efficient ultrasonic device, which uses a flexure-mode instead of an axial-mode transducer, is described in patents US 6322532 B1 and EP1089788 A1. The device is smaller than the conventional systems and particularly useful in transdermal and mucosal membranes, wherever relatively low power is required [55, 56].

A recent patent [57] describes a dental ultrasonic drug delivery device able to release an active into a target site, such as a root canal or a periodontal region, to treat periapical lesions or dental caries. The device contains a therapeutic probe which is inserted in the site to be treated and ultrasounds are then emitted with a frequency ranging from 800 KHz to 2 MHz. The system is capable of accurately cleaning the inside of root canals and killing bacteria. The drug is delivered in a mixed state with nanobubbles.

Recently, a periodontal gene delivery method, based on ultrasound and nano-/microbubbles, has been described. The method is aimed at transfection in periodontal tissue for gene therapy. The approach may be beneficial as it has minimal invasiveness and regional targetability, facilitating gene therapy for periodontal disease involving alveolar bone resorption [58].

With the aim of developing innovative and improved sonophoresis microdevices, further work is currently in progress to design and attain new fabrication techniques, biocompatible materials and simulation methods.

Although sonophoresis has been primarily used to enhance drug passage through the skin, it could also be applied to the delivery of actives through other keratinized tissues. In mucous membranes, the level of keratinization, and therefore the permeability barrier, is not as vast as in the skin stratum corneum. Probably due to this feature, the potentialities of the ultrasound technology have not been fully established in mucosal tissues. However, sonophoresis could be extensively studied to develop and employ this technique as an alternative method in transmucosal drug delivery.

5.3 Iontophoresis

To overcome the inherent shortcomings of limited depth of drug penetration and increase local and/or systemic drug delivery through epithelial tissues, iontophoresis has been extensively studied during the last two decades.

The phenomenon of iontophoresis has been known since 1900 [59]; currently, the typical area of its application in medicine is transdermal drug delivery, but it may also be used for enhancement of drug transfer through mucosal tissues [60–66].

Iontophoresis can be defined as "the permeation of ionized drug molecules across biological membranes under the influence of electrical current". The technique involves the transport into a tissue of ionic, charged and/or chargeable molecules under the effects of a direct or periodic electric field [67]. Nevertheless, it has been reported that iontophoresis does not contribute to rapid permeation of some molecules such as 5-aminolevulinic acid [68].

Ions in the presence of an electrolyte solution are transferred through the tissue by transient application of discontinuous electrical current using appropriate electrode polarity. Electrical energy assists the movement of ions across the biological tissue using the principle "like charges repel each other and opposite charges attract".

The technique implies the use of small amounts of physiologically acceptable electric current (1.0 mA/cm^2 or less) to drive therapeutic concentrations of charged drugs into the tissue. Several studies have demonstrated that drug permeation through oral mucosal tissues increases when the current density grows (Fig. 5.2) [60–62, 69, 70].

The application of electrical fields enhances drug delivery mainly through the paracellular pathway of biological membranes, thus promoting the transfer of hydrophilic drug ions [60, 61, 71, 72].

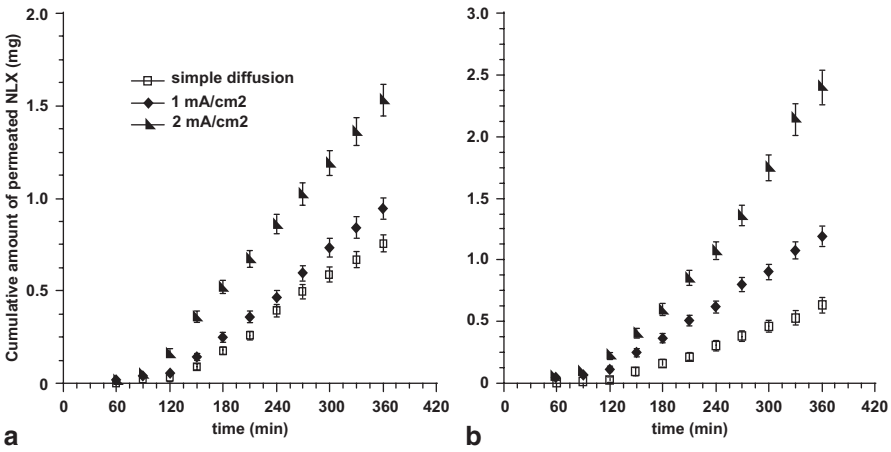

Fig. 5.2 Cumulative amount of naltrexone permeated across porcine buccal mucosa on simple diffusion and by iontophoresis versus time, using: as donor medium buffer solution simulating saliva (**a**) or natural human saliva (**b**), and as receptor-phase phosphate-buffered saline (PBS) simulating plasma [61]

5.3.1 Principles of Iontophoresis

Iontophoretic permeation must be considered as a combination of different mechanisms such as electromigration and electroosmosis, and taking into account the electrical properties of the membrane and the physico-chemical characteristics of the permeant [73, 74].

Electromigration describes the direct effect of the applied electric field on the charged species present in a formulation, whereby the transport of cationic drugs is enhanced from the anode compartment into the tissue and that of anionic drugs is promoted from the cathode [75].

Electroosmosis implies the movement of small sized cations (mainly Na^+), which generates a solvent flow that promotes the passage of non-charged molecules through the epithelium.

Anionic drugs are efficiently transported only by electromigration; in contrast, electroosmosis reduces the flux of anionic compounds. Cationic molecules are transferred by electroosmosis, and the rate of passage depends on the drug concentration in the biological flowing solvent. Nonionic substances may be dragged by the helpful promoting action of the solvent stream [76, 77]. Amphionic molecules are transported as a function of their isoelectric point and the environmental pH. Moreover, electromigration is useful for low molecular weight substances, whereas electroosmosis progressively contributes to the crossing of membranes for large molecules [73].

In particular, electroosmosis may be observed only if, in a system, electromigration of cations and anions is not symmetrical (e.g. in ion-exchange membranes). It has been reported that cationic drugs [75, 78] increase their mobility until keratinized tissues maintain the necessary total negative charge during the cation-exchange process.

Non-keratinized mucosae exhibit a better water permeability than keratinized ones, thus representing a suitable area for iontophoretical delivery of hydrophilic agents and cationic drugs. This area may also be a more convenient local site for electro-osmotic administration of neutral substances.

During the application of iontophoresis on the buccal mucosa, new hydrophilic pathways are created mainly by electrically enhanced solvent flux which accompanies the movement of cations and temporarily loosens the tissue structure [79].

The drug concentration has a crucial impact on iontophoretic flux and depends on the vehicle composition [75, 80, 81]. Proportionality between flux and concentration is usually observed when competing co-ions are present in the donor solution [82–84]; nevertheless, for several cations, it has been reported that iontophoretic fluxes do not increase proportionally with their concentration [78, 85, 86]. As an explanation of this behaviour, possible ion–tissue interactions have been invoked that could result in a progressive neutralization of negative charges of biological membranes. In line with this reasoning, the low flux measured at a high drug concentration could be caused by a progressively reduced electro-osmotic contribution.

Because of the complex nature of iontophoretic delivery, a number of attempts have been made to define the rate of drug passage through the epithelia. To predict the behaviour of electrically assisted drug permeation, several models of iontophoretic delivery are available.

5.3.2 Factors That Could Affect Iontophoretic Transport

Many factors could affect iontophoretic drug transport through biological membranes. Some factors are associated with the physico-chemical characteristics of the drug (e.g. charge and molecular size); other factors are connected to the drug formulation (e.g. type of vehicle, pH, viscosity, drug concentration and presence of other ions) and membrane biological properties (e.g. origin, age and sex). Also temperature, the equipment used, type of current and range and duration of iontophoresis could affect drug transport [87–89]. The aptitude of drugs to cross biological membranes is described by the permeability coefficient which is dependent on molecular size; an increase of molecular size implies a decrease of the permeability coefficient.

Usually, iontophoresis is conducted at the pH of physiological fluids that contain small co-ions more mobile than the drug-ion. As a consequence of ionic competition, the drug permeation through the tissue could be slowed down. The pH of drug formulation should be optimized to reach maximum drug ionization. In iontophoretic conditions, the steady-state flux is due to drug ion movement and it will be affected by the drug concentration.

Continuous direct current (DC) could induce membrane polarization, thus reducing the efficiency of iontophoretic drug delivery. To overcome this potential drawback, it is advisable to use the periodic delivery of DC current (pulsed). During the off time, the membrane depolarizes and returns to its initial status.

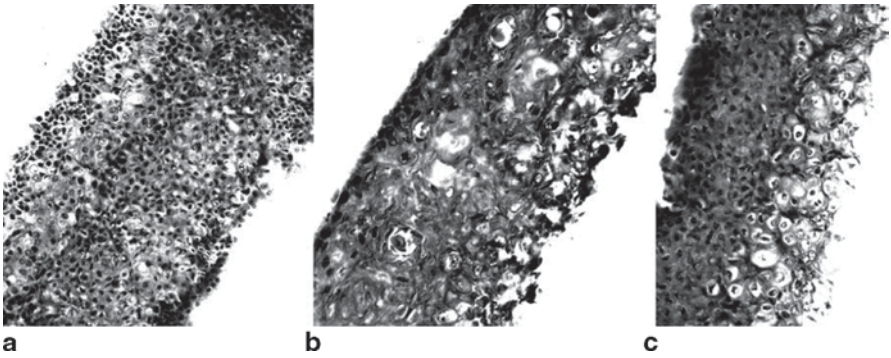

Fig. 5.3 Microphotographs of formalin-fixed, paraffin-embedded, haematoxylin-and-eosin-stained cross sections of reconstituted human oral epithelium: **a** control untreated; **b** sample exposed to drug permeation, enhanced by the application of 1 mA/cm^2 current density; cells appear vacuolated due to the accumulated intracytoplasmic drug; **c** sample exposed to drug permeation, enhanced by the application of 2 mA/cm^2 current density; superficial cellular disarray of cytopathic effects appear [61]

5.3.3 Effects of Iontophoresis on Mucosal Tissues

It has been reported that in cultured buccal tissues, following drug passive diffusion, even at high concentration a uniform cellular swelling in the absence of other significant changes in cell morphology or tissue structure has been experienced. In detail, cells appeared vacuolated due to the presence of intracytoplasmic drug accumulated in the tissue [61].

Since application of current on mucosae could cause some changes in the tissue structure, potential histomorphological changes and tissue organization have been investigated. Following the application of iontophoresis, cytoarchitectural changes consisting in cellular disarray have been highlighted. No severe cytopathic effects have been described for mucosal specimens treated with current density up to 1.0 mA/cm^2. Increasing the current density, superficial cellular disarray, called "wave effect", was observed. The increase of cytopathic effects (e.g. nuclear pycnosis, diffuse signs of abrupt keratinization and loss of cellular alignment) were attributed to the increase of the current density to 2 mA/cm^2 or more. No apoptotic effects have been found up to 2 mA/cm^2 [61].

The effects of current density on buccal mucosa are shown in Fig. 5.3.

For in vivo applications, the interposition of a moist pad between the electrode plate and the membrane surface should be recommended for making a perfect contact and preventing any epithelium burns. The pad overcomes tissue resistance and protects it from absorbing any caustic metallic compound formed on the metal plate surface [90–92].

5.3.4 Prediction of Iontophoretic Enhancement

Following the application of electric fields, the overall flux through the membrane could be considered as the addition of different contributions. In particular:

$$J_{ionto} = J_p + J_{em} \pm J_{eo}, \tag{5.5}$$

where

J_p Is the inherent drug flux due to passive diffusion (i.e. in the absence of electric field)
J_{em} Is the flux due to electric current application (i.e. electromigration transport)
J_{eo} Is the flux due to electro-osmotic transport

Since the oral epithelium is stratified, the inherent drug flux due to passive diffusion may involve a combination of paracellular and transcellular passage. Hydrophilic drugs are transported by the paracellular route, whereas lipophilic compounds are generally transported through the transcellular pathway. At the steady state, the whole passive flux is:

$$J_p = J_{para} + J_{trans} = \frac{D_p \varepsilon}{h_p} c_d + \frac{D_t K_p}{h_t} (1 - \varepsilon) c_d, \tag{5.6}$$

where

J_{para} and J_{trans} Are the fluxes due to paracellular and transcellular passage, respectively
ε Is the area fraction of the paracellular route
c_d Is the concentration of drug in the donor compartment
K_p Is the lipid/aqueous partition coefficient
D_p and D_t Are the diffusion coefficients in the paracellular and transcellular matters, respectively
h_p and h_t Are the lengths of the paracellular and transcellular pathways, respectively [93, 94]

The contribution to the flux due to the electric current application, driven entirely by an electrochemical potential gradient, is described by the Nernst–Planck equation:

$$J_{em} = \frac{D_p z F c_d}{RT} \frac{d\psi}{dh_p}, \tag{5.7}$$

where

J_{em} Is the electromigration flux
z Is the ionic valence of the permeant
F Is the Faraday constant
R Is the gas constant

T Is the absolute temperature
ψ Is the electric potential at any h point in the membrane

This equation assumes that iontophoretic flux is entirely dependent on the electrochemical potential gradient (electromigration) and ignores the contributions of the current-induced, convective solvent flow (electroosmosis). To provide a correlation with the intensity of the applied current, Faraday's law could be taken into account [95]. Accordingly, the flux J_e assumes the simplified form:

$$\left[J_{em} = \frac{t_{nd} I}{Fz} \right], \tag{5.8}$$

where

t_{nd} Is the transport number of the drug
z Is the valence of the drug-ion
I Is the applied current intensity

The transport number, t_{nd}, is defined as the fraction of the total current transported by a specific ion, and is a measure of its efficiency as a charge carrier. The sum of the transport numbers of all the ions present during iontophoresis equals 1 ($\Sigma t = 1$), illustrating the competitive trend of electrotransport.

The knowledge of a compound's transport number allows prediction of the feasibility of its iontophoretic behaviour [96].

Taking into account also the ion mobility, μ, an expression for the transport number of a drug (t_{nd}) in a binary cation situation has been derived assuming:

- A homogeneous nonionic membrane
- No interactions or associations among ions in solution
- Independence of ionic charge and mobility [97]:

$$t_{nd} = \frac{c_d \mu_d z_d}{\sum c_i \mu_i z_i}, \tag{5.9}$$

where:

μ_d and μ_i Are the mobility values of the drug-ion and all the other ion species present in the environment
z_d and z_i Are the valences of the drug-ion and all the other ion species present in the environment
c_i Indicates the total concentration of ions species in the environment

In the absence of competing co-ions in the donor solution, the flux becomes dependent only on the diffusivity ratio of the counter-ion and the drug [80].

Convective solvent flow, defined as electroosmosis, comes to exist when electrical field is applied across a charged membrane and determines drug transport [97].

The electro-osmotic flux can be expressed as:

$$J_{eo} = c_d v, \tag{5.10}$$

where *v* is the rate of convective solvent movement.

As mentioned above, the overall iontophoretic flux is given by the contribution of all implied phenomena.

Finally, the cumulative expression of J_{ionto} is described by the following equation:

$$J_{ionto} = \frac{D_p \varepsilon}{h_p} c_d + \frac{D_t K_p}{h_t} (1-\varepsilon)\, c_d + \frac{D_p z F c_d}{RT} \frac{d\psi}{dh_p} \pm c_d v. \tag{5.11}$$

As an alternative, accordingly to Faraday's law:

$$J_{ionto} = \frac{D_p \varepsilon}{h_p} c_d + \frac{D_t K_p}{h_t} (1-\varepsilon) c_d + \frac{t_{nd} I}{Fz} \pm c_d v. \tag{5.12}$$

The same equation could be useful to determine mathematical models of iontophoretic drug delivery system applied on keratinized tissues, even if for transdermal drug delivery a more complete model has been described [99]. In particular, compared to non-keratinized mucosa, keratinized tissues include superior content of lipophilic domains that could affect iontophoretic transport of lipophilic ions, probably due to possible lipid–lipid interactions [100].

5.3.5 Electrodes

The electrodes used to apply the electric fields across biological tissues should be inert, work at low voltage, provide a stable environment for the drug and the added substances and minimise the local transport of toxic species through the tissue of interest [77, 100].

The use of Ag/AgCl as the active electrode is well accepted because inactive electrodes, such as carbon or platinum, induce proton production that causes tissue irritation and reduces stability, or affects drug delivery [74].

5.3.6 Experimental Protocol for Evaluation of Iontophoretic Permeation

The most common type of setup, functional to assess iontophoretic movement through mucosal membranes, uses vertical Franz-type diffusion cells as an open two-compartment model (Fig. 5.4) [61, 62, 92]; however, other types of cells have also been employed [60, 101].

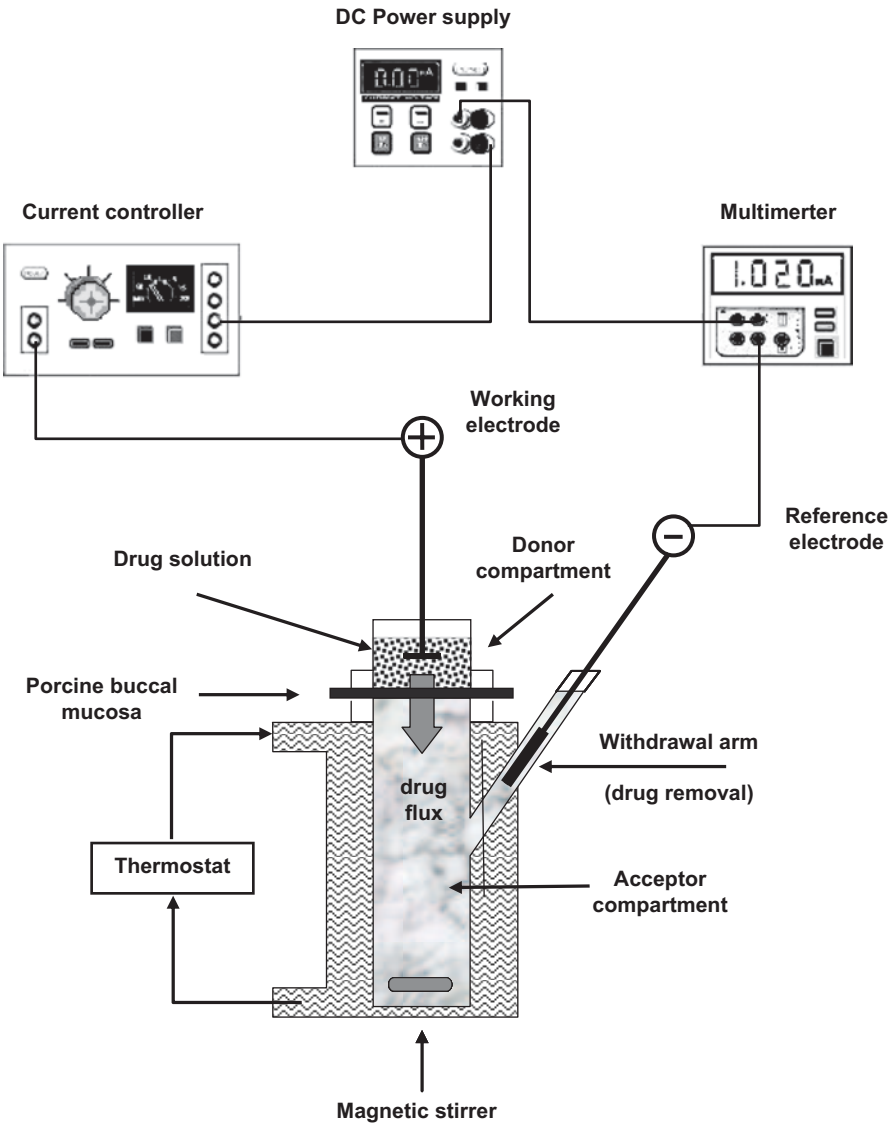

Fig. 5.4 Schematic experimental setup useful for ex vivo evaluation of the enhancement effects of iontophoresis on permeation through biological membranes

Usually, for *ex vivo* experiments, oral mucosal specimens are obtained by removing tissues from freshly slaughtered experimental animals. Different tissues from various animals including rabbits, dogs, monkeys, hamsters and pigs [102–105] as well as in vitro cultured tissues (e.g. reconstituted human oral epithelium) have been used as models for human mucosa [60, 61, 63].

Cell-culture models offer the advantage of highly defined, compliant systems in which experimental parameters and conditions can be easily adjusted. In particular, cultured human cell lines allow good correlations with in vivo behaviour, thereby avoiding the problems related to the use animal tissues from different species. However, when compared to other animal models, porcine mucosae have been considered the most representative model for human tissues.

Mucosal specimens could be surgically treated to remove excesses of connective and adipose tissue. As an alternative, specimens can be treated using the heat shock method. For heat separation of the epithelium, the mucosal tissues are dipped for approximately 1 min in saline solution warmed to 60 °C. Then, the connective tissue is carefully peeled off from the tissue to obtain the heat-separated epithelium. The connective tissue is completely removed and the epithelium remains along with the intact basal lamina. Heat treatment does not adversely affect the integrity of the mucosa [103]. The thickness of the tissues is measured using a digital micrometer. Slicing of the tissue with a dermatome is not advised since the preliminary freezing may alter the barrier properties of the mucosa [105]. After equilibration at 37 °C for 10 min, appropriate sections of mucosa are mounted in the diffusion cells.

In the donor compartment is placed the drug solution under an electrode of the same charge as the drug (working electrode); in the acceptor chamber are placed simulated plasma and a return electrode, opposite in charge to the drug (reference electrode). The electrode assembly is connected to a power source and the appropriate current density is then applied (Fig. 5.4). At regular time intervals, samples are withdrawn from the acceptor compartment and the sample volume taken out is replaced by fresh fluid. The drug transferred from the donor to the acceptor compartment is quantitatively determined [61, 62].

The electrodes are usually prepared using the method described by Jacobsen. Briefly, 10 cm of Ag wire is soaked successively in distilled water, ethanol, and fuming nitric acid and rinsed thoroughly with distilled water. Each process is performed three times for 3 s. The wire is then dipped into 0.1 N HCl, and a regular 1.0-mA current is maintained for 24 h using another Ag wire as a cathode to coat AgCl to the surface of the first Ag wire [60].

The use of platinum electrodes should be avoided since, during application, they may often be responsible for various redox reactions (data not published).

When reconstituted human oral epithelia, cultured on permeable polycarbonate inserts, are used as permeation in vitro models, inserts can be used as a Transwell two-compartment open model system (Fig. 5.5). On the apical side of the cell layers, representing the buccal environment (donor chamber), is applied a stagnant drug solution [61, 62, 106].

The acceptor chamber, representing the serosal side of mucosa, is filled with PBS to avoid cell stresses. The acceptor solution is stirred by means of a magnetic follower to avoid formation of stagnant boundary layers next to the membrane surface. Drug permeation could be evaluated following the above procedure.

Transepithelial electric resistance should be measured both at the start and at the end of each permeation experiment to have evidence of the barrier functionality of the membrane [107].

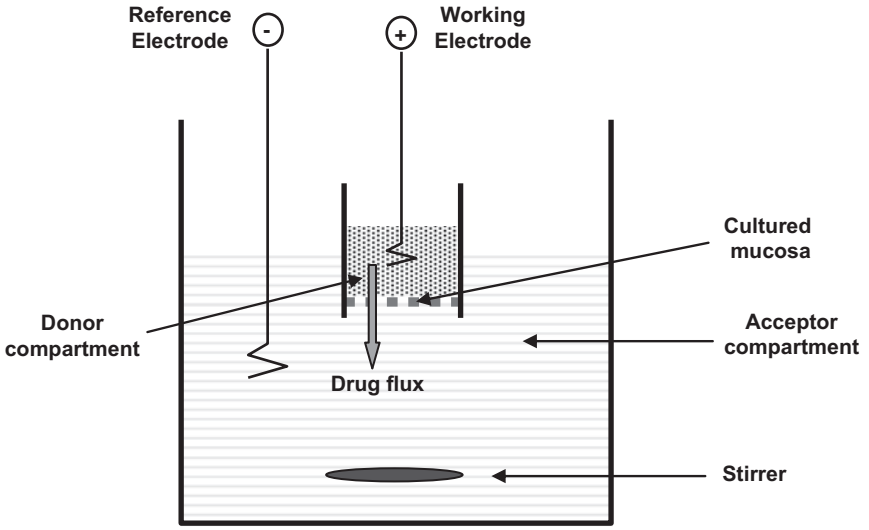

Fig. 5.5 Schematic experimental setup useful for in vitro evaluation of the enhancement effects of iontophoresis on permeation through cultured tissues

Table 5.1 List of drugs studied for oral mucosal iontophoretic delivery

Drug	Electric field intensity applied (mA/cm^2)	References
Atenolol HCl	0.1, 0.2, 0.3 and 0.4, alone	60
Salmon calcitonin	0.5, alone and combined with chemical enhancers	92
Ondansetron HCl	0.1, 0.2 and 0.3, alone and combined with chemical enhancers	70
Ropinirole HCl	1, alone	77
Lidocaine HCl	0.3, alone and combined with chemical enhancers	106
Lidocaine HCl	0.3, alone and combined with chemical enhancers	69
Nicotine hydrogen tartrate	0.1 and 0.3, alone and combined with chemical enhancers	69
Nicotine hydrogen tartrate	0.3, alone and combined with chemical enhancers	106
Diltiazem HCl	0.1 and 0.3, alone and combined with chemical enhancers	69
Naltrexone HCl	1 and 2, alone	61, 62
Naltrexone HCl	2, intraoral prototype device, in vivo trial	64
Galantamine HBr	0.5, 1 and 2, alone	63
Galantamine HBr	2, intraoral prototype device, in vivo trial	65

In Table 5.1 is reported a list of widely used drugs studied for oral mucosal iontophoretic delivery.

5.3.7 Reverse Iontophoresis

Currently, iontophoresis is referred to as a relatively new technology that promotes permeation, of both charged and neutral molecules through biomembranes, following the application of low levels of current. In recent years, it has been shown that current can also be used to remove molecules from the body. Reverse iontophoresis is the process in which molecules can be extracted from biological tissues by an electrically assisted process. In this process, whenever epithelial tissues are involved, the passage of substances from the body to the epithelium or from the epithelium out of the body could be promoted. Solvent flow (electroosmosis), generated during the process, is able to convect neutral molecules, therefore allowing their withdrawal from biological matrices with enhanced flux [108].

The application of reverse iontophoresis is useful for an accurate, continuous and non-invasive monitoring of homeostatic deviations of key metabolites, and it is becoming the primary objective of biosensor technology in various biomedical applications as well as in long-term or chronic therapies.

Although the available technologies that use non-invasive systems able to harvest marker molecules from the blood have had varied success and benefits, reverse iontophoresis could become the method of choice.

The proof of the concept of reverse iontophoresis is given by the gap of time, also called “off time”, during which a tissue, after the application of an electric field, self-depolarizes and goes back roughly to the initial electric condition.

The use of DC has been reported to cause adverse effects (e.g. electric burns, stinging or erythema) as a result of tissue polarization, so that the duration of DC application is limited to a period up to 15 min. The use of pulsed DC (or alternative current, AC) has been studied as a replacement to DC employment [109]. To avoid adverse effects in clinical situations, the application of AC with reversal of electrode polarity every 15 min is recommended; under these conditions, tissue irritation may be minimised [110].

Reverse iontophoretic extraction of glucose, theophylline and clonidine was described for the first time in 1989. The potential for non-invasive glucose monitoring was then demonstrated by both in vitro and in vivo experimental assessments [108, 111–114].

For diagnostic purposes, reverse iontophoresis has been successfully established to extract from the body various molecules, among which are: amino acids [115], lactate [116], lithium [117], phenytoin [118], glucose [113], amikacin [119] and valproate [120]. Caffeine and theophylline are often administered to premature neonates so the feasibility in their monitoring, using reverse iontophoresis in neonatal skin, has been reported. However, the amounts of drugs extracted were equivalent to those obtained by passive diffusion. In these circumstances, the benefit of the applied electric field is lost. In the case of neutral molecules, the incomplete functionalities of the skin barrier of premature neonates preclude the benefit offered by reverse iontophoresis. In contrast, for ionized species, where the principal iontophoretic transport mechanism is electromigration, the approach should be valid [121].

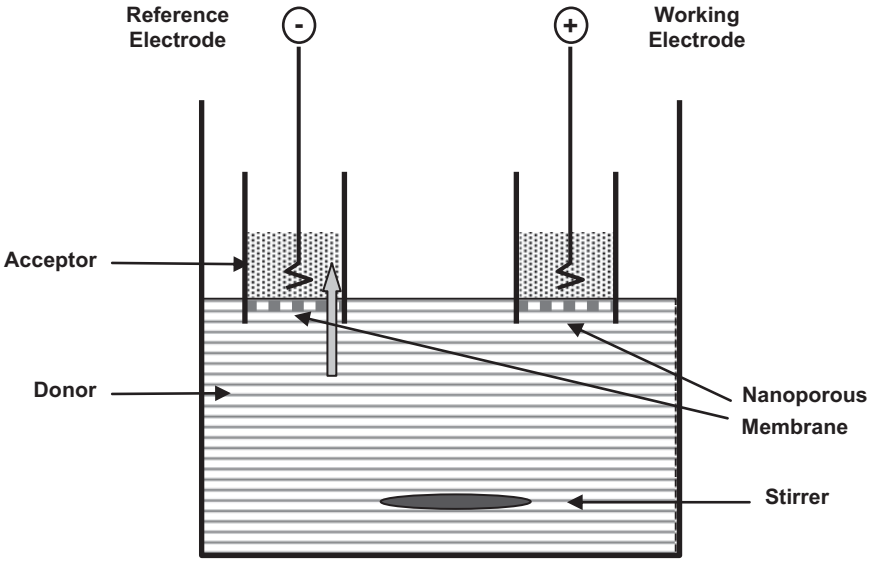

Fig. 5.6 Schematic setup for in vitro experiments with reverse iontophoresis

Non-invasive monitoring of urea for patients with chronic kidney disease has been established using a reverse iontophoresis controller device. The technique is able to track blood urea changes on dialysis [122, 123].

The effects of reversing the polarity of electrodes in iontophoresis has been investigated also to get a better transdermal absorption of peptide drugs (e.g. insulin and calcitonin) and to reduce dermal irritation due to iontophoresis. When the polarity of electrodes was reversed at intervals of 20 min for insulin and 25 min for calcitonin, drug absorption was effectively enhanced. The addition of urea to the insulin solution together with the switching technique brought about a remarkably facilitated absorption of insulin [124].

At the beginning of 2001, the Food and Drug Administration (FDA) approved an innovative sampling tool device. This approval has validated reverse iontophoresis in the medical field. According to FDA information, the device is described as a wristwatch-like glucose monitoring system that took readings through the skin every 20 min for up to 12 h, and is used to track trends in glucose levels over time. To pull glucose through the skin, the device uses a low electric current, so it is minimally invasive. A built-in alarm alerts the patient when glucose levels are severely low or high [125]. The alarm has the potential to increase the safety of diabetes management in children since it is able to notify dangerous nocturnal variations of glucose levels. The device is well tolerated by children and, compared with other standard methods, significantly improves glucose control [126].

Various examples of devices that use the technique of reverse iontophoresis have been described, patented and marketed [127–130]. A simple experimental apparatus to test the ability of reverse iontophoresis to extract molecules from a donor medium is shown schematically in Fig. 5.6 [131].

Experiments are performed using diffusion cells where both electrode chambers are located on the same side of a fluid surface from which the contained material should be extracted. The lower chamber (donor) contains both the substance to be monitored and an electrolyte solution. Each electrode chamber contains an Ag/AgCl electrode surrounded by an electrolyte solution. An iontophoretic current is then applied and, if required, the polarity of electrodes can be reversed at appropriate time intervals. The entire content of the electrode chambers are removed at the end of the experiment to quantify the amount of analyte extracted.

5.3.8 Devices Proposed for Iontophoretic Delivery in the Oral Cavity

Iontophoresis devices are already commercially available on the market for delivering drugs through the skin; despite the large interest in application of iontophoresis to the oral cavity, no devices are marketed for this purpose.

The US 2006/0161097 A1 patent application reports a device for iontophoretic administration of charged drug to tissues of the oral cavity. The device includes an applicator able to access any point in the oral cavity (e.g. a clamp-shaped sponge) and an external electric-current-generating element. The sponge, which contains the electrodes and allows contact with the oral tissue, is loaded with a suitable formulation containing the charged drug (e.g. lipid nanospheres, charged nanoparticles). The current, when applied, passes through the sponge in a direction normal to the surface of the tissue of the oral cavity and promotes drug permeation [132].

The key factor that determines difficulties in designing the delivery devices is the location of the electrode set. Both electrodes could be positioned in the mouth and, as a second choice, the donor electrode may be positioned in the mouth with the acceptor on the external side of cheek. On the basis of a computational model, for better mimicry of the real structure and conditions of in vivo drug release, it seems that the opposite location may increase the current efficiency of drug transfer. The computational model takes into account saliva film, mucous, mucosa, connective tissue and submucosa [79, 133].

Two recent patents describe an iontophoretic device which can be stably applied in the oral cavity to release ionic drugs via the oral mucosa over a long period of time. The device includes a power source, a working electrode connected to the power source and a non-working electrode as the counter electrode, all located within a support constituted of an artificial denture [134, 135].

An invention directed toward controlled drug delivery in the oral cavity is reported in the US 2004/0158194 A1 patent application. The invention refers to a controlled drug delivery device that is inserted into the oral cavity like a prosthetic dental implant and contains a drug reservoir refillable or replaceable as needed. The drug delivery may be passive or electromechanically controlled and may be accomplished by any one of the following mechanisms: in accordance with a preprogrammed regimen, delayed, pulsatile, chronotherapeutic, closed-loop, responsive

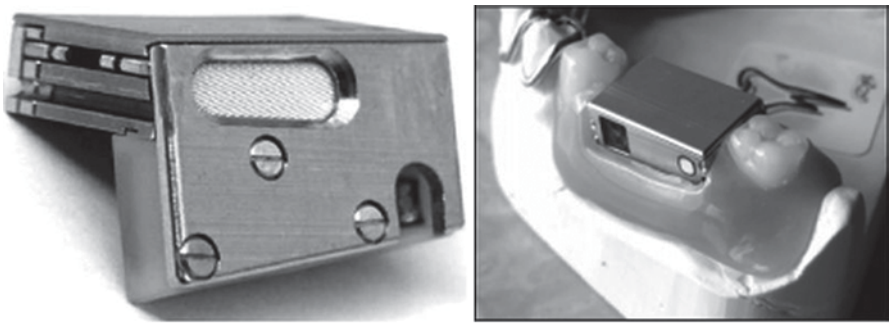

Fig. 5.7 The IntelliDrug system: **a** view of the device from the lingual side; the inlet port for saliva entry is recognizable; **b** placement of the device; the outlet port for drug solution (next to buccal mucosa) and the coaxial electrode ring (around the outlet for iontophoretic applications) are recognizable

to a sensor's input, on demand from a personal extracorporeal system, delivery regimen specified by a personal extracorporeal system. If needed, drug absorption in the oral cavity may be assisted or induced by iontophoresis [136].

Recently, this patent has been implemented by researches performed as a part of the IntelliDrug Project (supported by the European Commission under the 6th Framework Program) which was aimed to develop a controlled intraoral drug delivery device equipped with an electronic- and software-driven system. The device consists of a stainless-steel, two-molar-sized intraoral module containing an osmotic pump as a driving mechanism, a drug reservoir, an actuation mechanism for pushing the drug solution, an electronically controllable valve, a drug level sensor for monitoring the remaining amount of drug, a flow sensor for sensing the amount of drug administered, a power source and a subsystem for iontophoretic delivery enhancement. Following this mechatronic approach, the highly integrated, so-called IntelliDrug system has been described. The system is the size of two mandibular molar teeth (Fig. 5.7) and was developed to circumvent drug absorption through the gastrointestinal tract and the associated disadvantages.

On the lingual side, water from saliva enters the system through a water-permeable membrane (osmotic pump) and reaches the drug pill stored in the reservoir compartment. The resulting drug solution is pushed out of the drug reservoir and flows through a microfluidic duct.

A schematic overview of the IntelliDrug system is shown in Fig. 5.8.

A flow sensor allows the metering of both the flow rate and the concentration. The device incorporates an iontophoretic delivery enhancement by a coaxial electrode ring around the outlet. The rate of drug release is governed by the on-board electronics able to modify the duty cycle of the valve openings [137–141] (Fig. 5.8).

Drugs can be efficiently administered via the buccal mucosa by means of the IntelliDrug system. The device is intended to find application with various diseases.

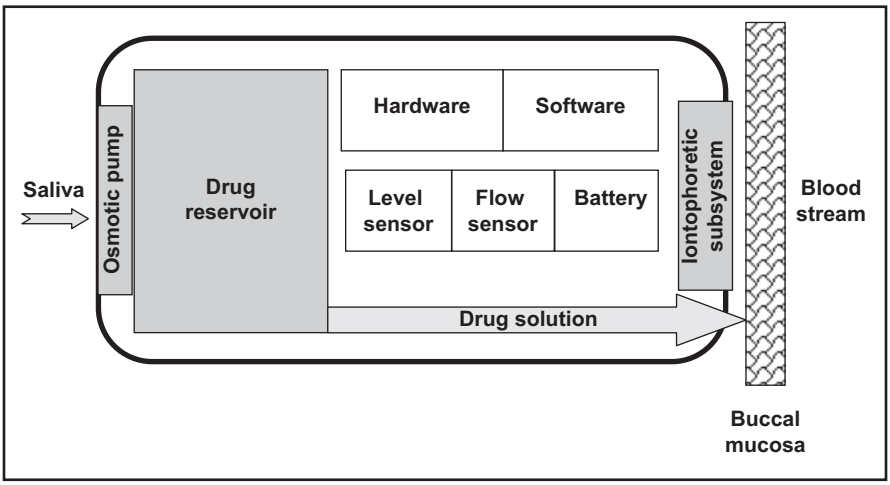

Fig. 5.8 Schematic overview of the IntelliDrug device

During in vivo trials on pigs, the usefulness of a prototype of the IntelliDrug system has been established for the transbuccal delivery of naltrexone and galantamine both in the presence and in the absence of iontophoresis [64, 65, 142]. The galantamine plasma levels are reported in Fig. 5.9 following buccal delivery by a prototype device in comparison to intravenous delivery.

Comparable results have been attained on humans (data not yet published).

Even if further studies are required to improve the system, the IntelliDrug device could be a revolutionary approach to drug administration.

5.4 Electroporation

To improve local and/or systemic drug delivery, penetration through biological membranes, as well as by iontophoresis, could be assisted by the electroporation technology.

The use of electropermeabilization, as a method of enhancing diffusion across biological barriers, dates from mid-eighteenth century [143].

The technology has been mainly used to enhance the skin permeability of molecules with different lipophilicity and size (e.g. small molecules, proteins, peptides and oligonucleotides) even for molecular weight greater than 7 kDa [144]. Its application to skin has been shown to increase transdermal drug delivery by several orders of magnitude.

Electroporation involves the application of high electric field pulses, ranging from 10 to 1000 V/cm^{-1} for a very short period of time, typically microseconds to milliseconds, to induce membrane perturbation. The increase in permeability is attributed to the induced transient formation of nanoscale defects (pores) in the membrane.

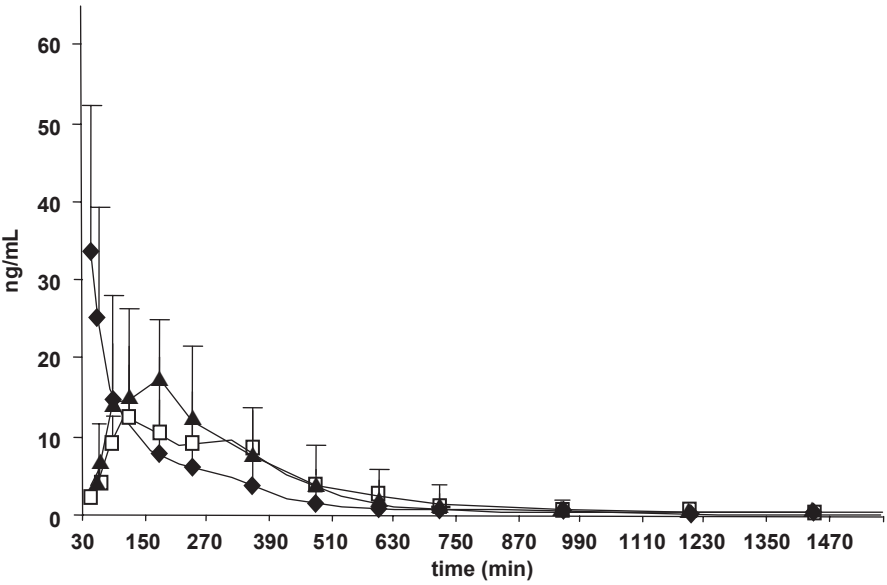

Fig. 5.9 Galantamine plasma levels (ng/mL) following in vivo delivery on pigs by a prototype of the *IntelliDrug* device. ♦ i.v. injection; □ buccal delivery; ▲ buccal delivery after application of iontophoresis

The increase in transport is attributed to a combination of different mechanisms (e.g. diffusion, electrophoretic movement and electroosmosis). Polarization is one of the basic mechanisms of membrane interactions with applied electric fields that generate accumulation of charges at the membrane surfaces. Due to restricted motion of ions, the interactions develop forces that can induce movement of particles inside the membrane. This motion implies structural rearrangement or fracture in the material, membrane poration and formation of new aqueous pathways (electropores) consequent to perturbation of the lipid bilayers of membranes. The pores then permit the molecules to readily cross the barrier, with a consequent transmembrane high flux. The pores are believed to be small (<10 nm), sparse (0.1 % of surface area) and generally short-lived (μs to s).

Diffusion is enhanced both during pulses and after pulses. The transport of charged molecules during pulses is electrically driven and is attributed to electrophoretic movements and very slightly to electroosmosis. In contrast to iontophoresis, the contribution of electroosmosis during high-voltage pulses is low [145]. It seems that a rise in temperature during current application plays a role in membrane permeabilization [96, 146].

The applied voltage, the time of exposure and the total number of pulses given can be adjusted to optimize the drug flux. However, similarly to iontophoresis, the application of the electroporation technique could also cause membrane damages as a consequence of high electrical current.

Electroporation can be reversible and irreversible. Reversible electroporation implies only a temporary increase in permeability: The cells, that undergo the pulsed electric field, survive.

In irreversible electroporation, the electric field is so high that the treatment may lead to necrosis and cell death. As a consequence, permanent permeabilization of the membrane is observed. Irreversible electroporation has applications in food industry, for sterilization and in medicine for tissue ablation [147].

Besides the electrical parameters, the physico-chemical properties of both the drug and the delivery environment could affect the transport by electroporation [148]. The key properties are the p*K*a of the drug and the pH of the delivery solution that are basic parameters influencing ionization of the molecule to be delivered. However, neutral molecules are also enhanced by electroporation, due to passive diffusion through the permeabilized membrane. The increase of the drug lipophilicity could decrease the enhancement magnitude. A further physico-chemical property of the drug, influencing the transmembrane transport by electroporation, is the molecular weight. It has been established that an increase in drug molecular size causes a decrease in transmembrane transport. The use of iontophoresis in conjunction with electroporation can expand the technique to the delivery of large molecules [145].

During electroporation, transmembrane transport could be affected by drug concentration: The increase of concentration promotes the transport rate. However, a non-linear relationship between drug amount delivered and drug concentration in the reservoir has been reported [149]. The choice of drug concentration in the reservoir could allow control of drug delivery.

Even if electroporation is used alone or in combination with iontophoresis mostly to enhance skin penetration, this technique has also been explored for delivery into other tissues and could be usefully employed also in transmucosal drug delivery.

Various examples of electroporation devices have been described, patented and marketed [150–153]. In particular, an interesting device uses an electrode microneedle plate to apply the electric potential to mucosal cells [154]. Electroporation could be a promising alternative as a non-invasive delivery of drugs. Combined with other enhancing methods, electroporation can provide modulated and adequate delivery also of macromolecules, according to the treatment.

5.5 Combinations of Enhancing Methods

The application of physical methods, able to improve permeation through the mucosae of the oral cavity, could represent a promising and valid alternative in drug delivery as they are safe and non-invasive. Although all these techniques have been individually shown to enhance drug transport, combinations amongst them have often been found more effective than each alone. Moreover, combinations of physical methods with other enhancing means can provide a modulated, more accurate and adequate delivery also for macromolecules and poor water-soluble compounds, according to the treatment to be undertaken.

5.5.1 Sonophoresis and Chemical Enhancers

It has been reported that, when applied simultaneously, chemical enhancers and therapeutic ultrasound have synergistic effects on drug delivery. Various concomitant phenomena could be invoked. Bilayer disordering agents, such as linoleic acid and ultrasound, determine the fluidification of lipid bilayers or create a separate bulk oil phase. The difference in diffusivity of solutes in either fluid bilayers or bulk oil phase is superior for bigger solutes, therefore producing greater enhancement for larger solutes [155, 156].

It has been observed that, in the absence of surfactants, the threshold ultrasound energy for giving a detectable change in skin impedance is about 141 J/cm^2. The addition of 1% sodium lauryl sulphate to the solution decreases the threshold to about 18 J/cm^2 [34].

Moreover, a combination of ultrasound with sodium lauryl sulphate leads to modification of the pH profile of the tissue. This change of pH within the microenvironment can affect both the structure of the lipid layers and the activity of the enhancer, thus promoting drug transport of hydrophilic molecules and macromolecules [157, 158].

The effects of chemical enhancers coupled with low-frequency ultrasound on the transdermal permeation of tizanidine hydrochloride were investigated. A synergistic effect was noted when sonophoresis was applied in the presence of different chemical enhancers. Formulation with the application of ultrasound may be useful in the development of transdermal therapeutic delivery systems of tizanidine hydrochloride [159].

5.5.2 Sonophoresis and Iontophoresis

The advantages of this combination include the fact that ultrasound and iontophoresis enhance drug transport through different mechanisms, thus making this combination rational. The limitations of this method include the possibility of requiring a relatively complex device compared with ultrasound or iontophoresis alone. After combining low-frequency ultrasound with iontophoresis, a synergistic effect on permeation of sodium nonivamide acetate has been observed. The combination offers significant enhancement of transdermal flux over either of the methods alone [160]. A similar behaviour has been observed using vitamin B12 as a model drug [161]. Since the drug is predominantly transported through shunt routes during iontophoresis, pretreatment with ultrasound could expand this effect resulting in synergistic enhancement.

5.5.3 Sonophoresis and Electroporation

The combination of ultrasound and electroporation may not be very promising due to the similar mechanisms of action. However, some synergy between ultrasound and electroporation has been reported. Simultaneous application of ultrasound enhances the transport of calcein and sulforhodamine and, at the same time, reduces the threshold voltage for electroporation [162, 163].

5.5.4 Iontophoresis and Chemical Enhancers

Although the use of iontophoresis results in much higher drug delivery if compared with conventional passive transepithelial delivery, it has some limitations. Chemical enhancers can be used in combination with iontophoresis to mitigate the resistance to cross the epithelial barrier and to facilitate drug diffusion.

The combination of the two techniques has been widely demonstrated on the skin (e.g. good permeation rates have been achieved for both small and large molecules or peptides like insulin) [164–166]. It has been reported that, in the buccal mucosa, optimization of the initial drug penetration into the tissue and reduction of lag time could be achieved by co-application of chemical enhancers and iontophoresis [69, 70, 92, 106].

5.5.5 Iontophoresis and Electroporation

These two techniques differ in several aspects such as the mode of application and pathways of transport; however, they can be used together for effective drug delivery. The combination of iontophoresis and electroporation is based on the difference between the mechanisms of action. Electroporation contributes to the disarray of the lipid bilayers of the membrane, thus creating new transport pathways and facilitating the passage of current during subsequent iontophoresis.

Studies on the enhancement effects of combined physical methods on sodium nonivamide acetate transdermal flux have been reported. The enhancement due to iontophoresis was higher than that observed following 10-min electroporation treatment.

Although there was no significant difference between the drug fluxes of iontophoresis combined with electroporation, and iontophoresis alone, the cumulative drug amount was significantly increased when treating with electroporation prior to iontophoresis. It seems that the application of a single electroporation pulse prior to iontophoresis yields 5–10-fold higher drug flux. This may indicate that electroporation accelerates the onset of iontophoresis [160].

Both the lipophilicity and the positive charges are important parameters able to affect the electrotransport of the drug. Understanding the effect of the physicochemical properties of the drug, as well as the electrical parameters, is essential for the optimization of transdermal drug delivery by a combination of electroporation and iontophoresis [148].

5.5.6 Electroporation and Chemical Enhancers

Synergistic effects of electroporation with appropriate chemical agents have been reported. Effective chemical enhancers for electroporation should stabilize the transient perturbation created by electroporation. The combination of these two methods probably expands aqueous pathways and prolongs the lifetime of the electropores [167, 168].

Using heparin, dextran sulphate, neutral dextran and polylysine as macromolecular enhancers, the influence on mannitol transport has been studied in vitro. Electroporation alone increased transdermal mannitol delivery by approximately two orders of magnitude. The addition of macromolecules further increased transport up to fivefold. It seems that these enhancers interact specifically with transport pathways created at high voltage. Although all macromolecules studied enhanced transport, those with greater charge and size were more effective [169].

Also the efficacy of electroporation in enhancing topical delivery of cyclosporine A can be further increased by pretreatment of tissues with chemical enhancers such as Azone and menthol [170].

5.6 Conclusions

Penetration enhancement techniques are gaining wide popularity as they are able to provide non-invasive and convenient means for local or systemic delivery of drugs that are characterized by a poor bioavailability profile, short half-life and multiple-dose scheduling.

In recent years, physical methods of penetration enhancement have been extensively investigated alone or in combination. The development of new delivery devices equipped with subsystems able to promote transepithelial drug permeation by physical enhancement methods in combination or not, in the near future, could allow new clinical applications.

Additional physical methods, extensively used to enhance drug permeation through the skin, despite being very promising have some infrequent applications on mucosae of the oral cavity. However, these further methods should be adequately developed and improved before extensive application in this area.

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Chapter 6
Characterization Methods for Oral Mucosal Drug Delivery

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6.1 Introduction

Research on buccal delivery systems has been ongoing for decades; however, despite several efforts, very few formulations have gained regulatory approval and appeared on the market. With the advent of the latest drug delivery technologies and assessment techniques, research on buccal delivery systems is taking new strides but it is important to adopt standardized evaluation methods for both in vitro and in vivo testing so that the data generated using these methods are meaningful. The purpose of this chapter is to compile and evaluate different evaluation methodologies in order to provide direction for future investigations related to buccal delivery of drugs and drug products. In this chapter, an overview of the studies that have been performed to characterize the buccal mucosa as an absorbing membrane is given. In addition, other characterization methods are described and discussed that facilitate the formulator in their decision on the optimal formulation such as pharmacokinetic (PK) studies, mucoadhesion studies, residence time evaluations, and dissolution testing.

6.2 The Barrier Nature of Oral Mucosa

The resistance offered by the buccal mucosa may be termed "the barrier," the characteristics of which are drug dependent. The nature of the barrier could be physicochemical or enzymatic. The resistance offered by tissues to the diffusion of drugs

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along the transcellular or paracellular pathways in the buccal mucosa can be considered a physicochemical barrier. The barrier is enzymatic, and can affect molecules that are prone to extensive enzymatic biotransformation.

The physicochemical barrier can be explained based on the structure of the mucosa. The buccal epithelium, especially the superficial one third to one quarter, is mainly responsible for its barrier nature [1], which is due to the materials that are released from the membrane-coating granules (MCGs) found in the epithelia. MCGs are oval or spherical in shape (100–300 nm in diameter) and contain a lipid portion within the membrane [2]. These lipids are extruded when the membranes of the MCGs fuse with cell membranes, and they serve as the primary barrier to the permeation of molecules across the buccal mucosa [3]. Because of the lipophilic nature of the barrier, lipophilic molecules can cross the buccal mucosa with relative ease when compared to hydrophilic molecules.

The buccal mucosa is nonkeratinized and relatively permeable to water when compared to keratinized epithelia, such as the skin. Neutral lipids like ceramides and acylceramides contribute to the barrier function of the keratinized epithelia. Nonkeratinized epithelia, on the other hand, contain only minimal amounts of ceramides but are rich in polar lipids such as cholesterol sulfate and glucosylceramides [4]. In addition, the buccal mucosa lacks the structural organization of lipid lamellae as seen in keratinized epithelia such as the skin [5] and this, therefore, results in relatively higher permeabilities to molecules.

A single structural element of the buccal tissue may not be the sole determinant of its barrier function. Apart from the epithelium, the basement membrane, the layer of mucus, and the salivary film each contribute to some extent to the barrier nature of the buccal mucosa.

The basement membrane consists of a continuous layer of extracellular materials and is present between the connective tissue and the basal layer of the epithelium. Charged molecules may interact with the basal lamina which may, act as a barrier to the passage of immune complexes [6], endotoxins [7] and insulin [8]. Although the barrier nature of the buccal mucosa can be primarily attributed to the superficial layers, the basement membrane acts as a barrier against the transport of macromolecular substances into the connective tissue [7, 9].

The mucus layer covering the epithelium might also act as a barrier for the absorption due to many anionic and cationic functional groups attached to the mucins. Mucins belong to a category of large, heavily glycosylated proteins [10], which are made of a protein core attached to an oligosaccharide chain [11]. Gel-like characteristics of mucins are imparted by the heavily glycosylated protein core. The water-holding capacity and resistance to proteolysis are due to dense coatings of mucins, which also contribute to the mucosal barrier [12].

The oral membranes are continuously supplied with fresh serous and mucous saliva. This saliva is secreted by the salivary glands, and forms a film (70–100 μm thick) that helps in maintaining moisture in the oral cavity [2]. The amount and composition of the saliva exhibits interindividual and diurnal variations. Furthermore, the presence of certain diseases or the administration of some medications can alter the production of saliva [13]. Salivary enzymes such as carbohydrases, esterases,

and phosphatases may also contribute to the degradation of exogenous compounds [14]. Although saliva per se does not contain peptidases, these enzymes are likely to be found in the oral cavity due to the complex bacterial microenvironment.

Enzymes like aminopeptidases, carboxypeptidases, dehydrogenases, and esterases that are present in the oral mucosal membrane may cause biotransformation of molecules administered in the oral cavity [14]. Protease is present intracellularly and hence these enzymes may not play a significant role in the metabolism of molecules that do not enter the epithelial cells [15]. Peptidases are present extracellularly on the surface of the buccal mucosa [16] and, hence, they act as significant barriers to the permeability of compounds that are susceptible to these enzymes.

6.3 Drug Transport Within the Oral Mucosa

Buccal absorption of drugs follow first-order rate kinetics. Drug transport across the buccal mucosa occurs mainly by passive diffusion. For some compounds, a carrier-mediated process may be involved. Regardless of the mechanism, generally, small molecules will be absorbed faster than large molecules but this is not an absolute rule. Dextrans with molecular weight up to 70,000 Da cross rabbit oral mucosa [17], but horseradish peroxidase (molecular weight, 40,000 Da) does not. Other specialized mechanisms, such as pinocytosis or phagocytosis, may not play a role because of the presence of stratified epithelium within the oral cavity.

6.3.1 Passive Diffusion

Passive diffusion is the primary mechanism of drug transport for the majority of permeates across the oral mucosa. This fact was first identified during a study of the buccal absorption of amphetamines [18]. In this study, the extent of absorption of amphetamines was found to be linear with time, indicating a passive process. Thereafter, many studies were conducted to demonstrate that drug transfer across the oral mucosa occurs through passive diffusion [19–23]. Tavakoli-Saberi and Audus [24] demonstrated the permeation of atenolol and alprenolol to be passive processes. Overall, the physicochemical properties of the drug and the buccal membrane together govern the transport rate of permeants that follow passive diffusion.

6.3.2 Carrier-Mediated Transport

Certain molecules need a carrier for their transport across the buccal mucosa. The buccal transport of sugars such as D-glucose and L-arabinose was shown to be stereospecific and saturable, indicating the existence of a carrier-mediated process [25, 26]. In addition, the transport of D-glucose across human oral mucosa is inhibited

by 2,4-dinitrophenol, phlorizin, and a sodium-free medium, which demonstrates the presence of a sodium-dependent transport system [26]. The transport of L-ascorbic acid across human buccal mucosa requires the presence of sodium, indicating that the process is carrier-mediated [27]. In a buccal absorption test, it was demonstrated that L-amino acids cross the buccal mucosa by a carrier-mediated process. The process was found to be stereospecific, with predominantly L-amino acids being transported. In experiments with L-methionine and L-leucine, the process was shown to be saturable. These amino acids appeared to have at least one common transport mechanism [28]. Utoguchi et al. reported an energy-dependent, carrier-mediated transport of monocarboxylic acids in rabbit oral mucosa [29, 30]. Similarly, it was demonstrated that the buccal transport of several other compounds such as thiamine, nicotinic acid, and nicotinamide is carrier mediated [27, 31, 32]. In addition, oral mucosal absorption of cefadroxil, a first-generation cephalosporin antibiotic, was reported to involve a special transport mechanism. Its absorption was saturable and was shown to be inhibited by the presence of another amino cephalosporin—cephalexin [33]. The uptake of other antibiotics like ciprofloxacin and minocycline across cultured epithelial cells (TR146) was also demonstrated to be saturable and competitive [34]. Finally, the buccal absorption of angiotensin-converting enzyme (ACE) inhibitors, captopril [35], lisinopril, and enalapril [36], does not follow the pH partition hypothesis and is demonstrated to be carrier mediated.

6.4 Methods to Study Absorption from the Oral Cavity

The assessment of the feasibility of developing a buccal drug delivery system often starts with an evaluation of whether the drug can permeate buccal membranes or not, and, if the drug permeates the buccal membrane, whether it is to be delivered at rates that provide therapeutic levels. Buccal drug absorption can be studied using in vivo and in vitro methodologies. In vitro investigations using excised tissues are complicated by the fact that absorption may not occur via passive diffusion, as described above. This raises the question during such studies: “To what extent are active transport mechanisms maintained in the excised tissue?” Tissue storage methods (between excision and the beginning of the experiment) and the storage medium become important considerations in order to preserve the viability of the buccal tissue. In contrast, in vivo methods do not carry the above-mentioned concerns since the tissue is alive. However, they have their own set of issues and precautions to be taken into account.

6.4.1 In Vivo Methodology

Initial investigations using in vivo techniques date back to as early as 1967. Beckett and Triggs developed an absorption test in human subjects [18] which was later

modified and improved by other investigators. The basic buccal absorption test and the later modifications are discussed in the following sections.

6.4.1.1 The Buccal Absorption Test

In the very basic version of a buccal absorption test, a measured quantity of a drug solution is introduced into the oral cavity of human subjects. The subject swirls the solution gently in the oral cavity for a predetermined period and carefully expels it. The oral cavity is then rinsed with drug-free buffer, and all the expelled solutions are pooled. Measuring the volume and the drug concentration of the combined expelled solutions will provide the information about the total amount of drug absorbed through the membranes of the oral mucosa. Although this test is easy to perform, there are significant disadvantages. For example, it provides no information about the amount of absorbed drug that is bioavailable. Drug loss could occur due to swallowing and this test fails to identify or correct for the loss of drug due to unintentional swallowing. In addition, variations in drug absorption across different regions of the oral cavity cannot be distinguished. Moreover, this test may not be an ideal one to study drug-absorption kinetics because a separate experiment is required for each time point. It becomes increasingly difficult for a subject to hold a solution in the mouth for longer times. Finally, because of the fact that pH of the solution within the buccal cavity changes constantly because of salivary secretions, the mean pH is considered for calculations.

6.4.1.2 Refinements to Buccal Absorption Tests

The method of Beckett and Triggs has been refined by various researchers. These refinements include the addition of a correction factor [37] and the inclusion of a nonabsorbable marker such as phenol red[38]. In order to calculate the drug's absorption kinetics, aliquots of the solution were collected from the oral cavity at predetermined intervals over 15–20 min [39]. This modification has added advantages over earlier modified tests (where corrections for saliva secretion, accidental swallowing, and changes in pH could be made) in that a complete absorption curve can be determined in a single test.

It is suggested that about 20–25 mL of drug solution be used for buccal absorption tests as this volume does not cause discomfort to human subjects. It also allows a homogenous mixing of contents in the oral cavity, and provides enough volume for the drug to remain in solution throughout the test [18, 40, 41]. In order to account for nonabsorbable losses such as unintentional swallowing, certain marker compounds are added to the drug solution [25, 42, 43]. Compounds like inulin [25], ^{125}I-labelled PVP [44], PEG [42], and phenol red[45, 46] have been used to assess the extent of drug loss arising from nonabsorbable sources. A few authors included pretest modifications such as cleansing the mouth [47], adjusting the pH [39], and warming the buffered drug solution to 37 °C immediately prior to the buccal absorption test [25].

Once the contents are expelled, the froth is allowed to settle down, or a small volume (0.3 or 0.5 mL) of buffer is added in order to compensate for the froth volume [48] or volumes are adjusted by weight [39]. If repeated tests are to be performed consecutively, a minimum period of about 15 min after a 5-min contact time; and a wait of 50 min following a 30-min contact time, is suggested [37]. Other authors prefer longer waiting times between repeats [39, 41, 49]. The time period depends on the physicochemical properties of the drug as well as the duration of the buccal absorption test. Longer waiting times must be allowed with more extensive drug contact or when very lipid-soluble drugs are investigated. It is evident from the successive recoveries of the drug after repeated rinsing (at intervals) following a single buccal administration test that the loss of drug from the oral cavity and its entry into the systemic circulation, do not occur simultaneously. In a buccal absorption study by Kates [50], it was reported that the half-life for the appearance of propranolol in the blood was about three times the half-life for its disappearance from the oral cavity, indicating that transfer of propranolol into the buccal membrane may be the rate-limiting step.

6.4.1.3 Limitations of Buccal Absorption Tests

As drug absorption takes place all over the mucosal surfaces, this method cannot identify the relative permeabilities of various regions of the oral cavity. There is also a continual and erratic secretion of saliva throughout the duration of the test. Depending on the volume of saliva that it contains, the pH and concentration of the drug solution change. In addition, saliva may interact with the drug resulting in possible interference with analytical procedures.

The buccal absorption test and its refinements are older techniques that are not popular at the present time. The lack of sophistication of the test and the involvement of human subjects (necessitating Institutional Review Board approval) are some of the reasons for this test falling out of favor. Nevertheless, the literature involving this test has been briefly described here for the completeness of the narrative and because there is a resurgence of interest in simple test procedures that give an indication of bioavailability. This interest occurs as a consequence of the fact that company pipelines are very limited and the novel drug delivery systems are increasingly coming into prominence. How does the researcher know that there is any propensity for the test drug to be absorbed by the non-oral route? If testing can be easily undertaken and is not prohibitively expensive, yet gives an indication of utility, it will guide formulation endeavors in a manner that is less costly and obtrusive than PK testing. Positive results will also give researchers the confidence to later undertake more expensive PK studies that will be definitive. It is possible that researchers will further refine this test in the future, making it a better way to guide formulation activities.

6.4.1.4 Perfusion Cells

Perfusion cells can be used to study buccal absorption in animals and humans. They are either clamped or attached to the respective mucosae within the oral cavity and the drug solution in question is perfused through the cell. The amount of drug that disappears from the perfusate is generally considered to be the amount absorbed through the mucosal membranes. This method has the advantage that the drug loss due to nonspecific absorption can be avoided. Furthermore, it provides valuable information regarding regional variation in drug absorption. The primary disadvantage with the perfusion cell is leakage of the drug solution from the cell and this loss can be assessed using a nonabsorbable marker compound in the perfusate.

Rathbone has designed an improved buccal perfusion cell that is devoid of leakage issues [21]. Intra and intersubject variations were minimal with this cell. A closed perfusion cell was developed by Barsuhn et al. [51] to investigate the buccal transport of flurbiprofen in human volunteers. This closed perfusion cell apparatus is considered to be a significant improvement over the in vivo buccal absorption test and disc methods (see below) to estimate the absorption rate of compounds across specific regions in the oral cavity. Buccal perfusion cells can avoid interference from salivary secretions and, therefore, the pH and temperature of the perfusate can be maintained constant. They are more informative when the appearance of drug in the plasma is simultaneously monitored.

6.4.1.5 Disc Methods

Using disc methods, investigators were able to study kinetic rates of transfer and drug loss across a fixed area of the oral cavity. The disc method, first developed by Kaaber [52], is a quantitative method that uses an airtight sampling chamber containing a disc of dry and ash-free filter paper overlaid with a disc of porous membrane material. Kaaber used this method to investigate the transmucosal transport of water and ions. Schur and Zeigler [53] used a polytef disc onto which a filter paper disc, previously soaked in water, is secured. The drug in its powder form is spread onto the filter paper and the disc is placed in contact with the mucosa. These disc methods are difficult to use and pose problems such as leakage of the drug from the disc and interference caused by saliva.

6.4.2 In Vitro Methods

Ideally, the evaluation of buccal delivery systems should be performed in vivo. However, this may not be possible, especially during the early developmental stages when there is a limited availability of safety information about the drug in question. In vitro experiments, therefore, are important to obtain early information on drug permeation. These methods use excised tissue or synthetic membrane in an

experimental setup where the membrane can serve as the barrier to the free movement of drug molecules. The challenge however is to find a biological or synthetic membrane that can mimic the actual barrier functions of the buccal tissue. To this end, membranes obtained from many different species of animals, both excised and cultured, have been investigated for their potential use in in vitro experiments. These membranes range in structure from keratinized to nonkeratinized and have varying permeabilities. Generally, membranes from animal sources tend to have lower permeabilities to drug compared with human buccal membrane, probably because of the keratinization of the former. The primary objective of in vitro permeation studies is to simulate diffusion conditions in man, thus reducing the requirement for in vivo experiments using animals or humans.

Currently, most in vitro studies investigating drug absorption through the buccal mucosa use buccal tissues from animal models. In a typical protocol, tissues are collected immediately after the sacrifice of the animals and transported to the laboratory in Krebs buffer (or other solutions) maintained at 4 °C. Initially, the buccal mucosa, along with the connective tissue, is isolated from the other tissues, such as muscle. Next, the connective tissue is removed from the buccal mucosa. Mucosal membranes are then preserved in ice-cold (4 °C) buffer (usually Krebs buffer) until used for in vitro permeation experiments.

6.4.2.1 Choice of Animal Species

The selection of species is made based on the structure of human buccal tissue. In order to obtain reliable data that can be compared with humans, experiments should be performed with nonkeratinized mucosa, e.g., from humans, monkeys, pigs, or dogs. Since rodent buccal tissue is keratinized, it is not comparable to human buccal mucosa.

It is ideal to conduct experiments using human buccal tissue. Apart from very small biopsies, human tissue is insufficiently available for large sets of in vitro experiments. For in vitro permeation experiments, cultured human buccal tissue can be used as a substitute.

As an alternative, researchers have used various animal models including the oral mucosa of rats [15, 54] and hamsters [55–57], but these mucosal membranes are keratinized. The buccal mucosa of the rat is very thick and so may not simulate the mucosal membranes of humans. Nevertheless, many investigators studied drug absorption using the keratinized epithelia of the hamster cheek pouch or rat buccal mucosa [58, 59]. The primary advantage of using these tissues is their relatively large availability.

Veillard et al. [58] performed in vitro studies in which the hamster cheek pouch was compared to dog tissue, mounted in a modified Ussing chamber. When corrections were made for tissue thickness, the nonkeratinized dog tissue appeared to be more permeable. Eggerth et al. [60] used hamster cheek pouch for in vitro absorption studies with dextromethorphan hydrobromide and the permeability was observed to be 25–30 times less than in dogs and rabbits. It was recommended that hamster cheek pouch should be avoided for buccal experiments because permeability coefficients for drugs in humans, and in hamsters, were found to be entirely different.

Tavakoli-Saberi and Audus [24, 61] developed a model where cultured hamster pouch buccal epithelium was used.

Rabbit buccal mucosa is another alternative for in vitro permeability studies. Although it is said to be nonkeratinized, there is a considerable degree of parakeratinization in rabbit buccal epithelium [1]. Further, there is a sudden transition from nonkeratinized to keratinized regions [62] making it hard to isolate the nonkeratinized regions. Therefore, the results based on these studies may not be extrapolated to humans [63]. The availability of nonkeratinized regions is also small and hence, it may not be practical to conduct a large number of experiments. The mucosal membranes of monkeys were also used for buccal permeation studies [64, 65]. However, this may not be a practical alternative because monkeys are expensive to maintain; their oral epithelium is thinner and consequently more permeable when compared to humans. Structurally and with respect to blood flow, canine tissue appears to closely resemble human buccal tissue [66]. Beagle dogs have been used for in vivo studies with peptides and diclofenac [67–69]. They have a thinner buccal epithelium compared to humans, resulting in higher permeability values.

Porcine tissue is available in large amounts from slaughterhouses. Pigs have anatomic, physiologic, and metabolic similarities with humans [70]. Porcine buccal mucosa is nonkeratinized and its composition closely resembles human buccal mucosa [4, 71–73]. Furthermore, the buccal mucosal thickness of humans and pigs is similar. Apart from the similarities in the structure and morphology, the permeability characteristics of porcine buccal mucosa resemble that of humans [73, 74]. Sattar et al. [75] reviewed the list of drugs that were investigated using porcine buccal mucosa as a model for transmucosal delivery. Histological evaluation of porcine buccal tissue revealed that the buccal epithelium remained viable up to 9 h postmortem, and using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, it was reported that the buccal epithelium remained viable for up to 12 h. Hence, when porcine buccal mucosa is being used for in vitro permeation experiments, it is recommended to complete the experiment within 9–12 h after animal sacrifice [76].

6.4.2.2 Tissue Preparation

In order to obtain epithelial membranes, the epithelial layer has to be separated from the underlying tissue and this can be accomplished chemically, surgically, or by using heat.

The chemical method involves separation of the underlying tissue using a splitting agent such as ethylenediaminetetraacetic acid (EDTA). Chemical splitting has the advantage that the epithelium can be obtained in its entirety. However, it is not certain to what extent the barrier functions of the mucosa are compromised following chemical treatment. The basal lamina is attached to the connective tissue [72] and may become separated during tissue preparation and will, consequently, be excluded from the diffusion experiments. Surgical separation can be used to isolate the underlying tissue either using scissors or dermatoming the upper part

of the mucosa [46, 77]. The advantage of this method is the fact that the barrier is not damaged chemically and that the basal lamina is included. The tissue needs to be dermatomed fairly thick and, by doing so, a large amount of connective tissue is included, resulting in thicker sections. When heat is used as a means to isolate the epithelium from the underlying tissue, the tissue is placed in a buffer (usually Krebs) which is exposed to 60 °C for 1 min. Then the epithelium is peeled inward.

6.4.2.3 Apparatus

Several types of diffusion cells are used for in vitro experiments. The buccal epithelial membranes or the whole mucosal membranes are mounted with the epithelium facing the donor compartment. Since some workers felt that the permeability of the buccal epithelium resembles that of the whole buccal tissue, the whole tissue is often used during diffusion experiments.

Diffusion Cells: There are different types of diffusion cells such as the standard Franz diffusion cell [78–81], flow-through diffusion cell (modified Franz diffusion cell) [82], and Ussing chambers[83–85]. Using these cells, the total amount of drug diffused through the tissue in a specific period and the rate of drug diffusion can be determined. The latter cannot be determined using perfusion cells. The epithelial tissue is sandwiched between the chambers and the prepared film or drug formulation is applied on this tissue. The standard Franz diffusion cell can be described as a static cell which has a cell cap that opens upwards facing the air and the receiver chamber contains simulated saliva or other buffer that can maintain sink conditions. Prepared formulation film is applied on the epithelial membranes or whole mucosal membranes mounted tightly between the chambers to prevent leakage. The temperature is maintained at 37 ± 0.5 °C and a stirring bar is used to mix the solution in the receiver compartment. Aliquots of the receiver are collected at preset intervals and replaced continuously. The primary disadvantage with standard diffusion cells for an in vitro permeation study is that the volume of the receptor compartment is limited. This may not be a problem for drugs that are fairly soluble, but with poorly soluble drugs, sink conditions may not be maintained. This disadvantage may be overcome by the addition of a cosolvent such as ethanol or polyethylene glycol, or by the use of a surfactant in the receiver phase [86]. However, the addition of cosolvents may have implications for the permeability of the isolated mucosa. Another approach to address this problem is to avoid building up of the drug in the receptor. This can be done by continuously removing the receptor fluid and is the basis for the modified Franz diffusion cells or the flow-through diffusion cells [60–62].

Ussing chambers are the most widely used two-chambered cells with donor and receptor compartments separated by a biological tissue. The donor is loaded with the drug dissolved in a physiological buffer such as Krebs-Ringer, and an equal volume of buffer is placed in the receptor chamber. Stirring in the chamber is maintained by pumping carbogen gas and the buffer helps in maintaining the viability of the buccal tissue. Samples are collected from the receptor chamber at predetermined intervals, analyzed to estimate the amount of drug permeating from the donor to the receptor, and the results are plotted as a function of time.

6.4.3 Limitation of In Vitro Studies

Although in vitro techniques offer an excellent means to study the absorption of molecules across the oral mucosal membranes, it may not always be possible to correlate the data with those obtained from in vivo results. Therefore, careful consideration must be given to the conditions under which the experiments are conducted, and adequate measures must be taken to minimize artifacts. Every possible care must be taken to avoid experimental conditions that do not represent the in vivo situation and, similarly, factors that impact in vivo results must be included in the experimental design, as far as practical.

The thickness of the buccal tissue is an important factor in in vitro permeability experiments and either the buccal epithelium itself, or the full thickness membrane may be used in the experiments. Though the upper epithelial layers are responsible for the barrier function, it has been demonstrated that the inclusion of connective tissue may affect the buccal permeation of molecules [87]. Since the connective tissue is hydrophilic, lipophilic molecules are affected the most when connective tissue is included in the in vitro experiments. Therefore, it may be more meaningful to use the epithelium alone. Since the microvasculature is present immediately beneath the epithelial surface, this method avoids the artificial resistance offered by the connective tissue.

In many instances, the mucosal membranes are isolated from cheeks collected from animals prone to naturally occurring tissue damage from mastication. Tissue damage may enhance drug permeation. There may also be unintentional damage to the membrane during isolation surgically, or by other means. This problem could be detected by the use of integrity markers. The most common method to confirm tissue integrity involves the inclusion of a non-absorbable marker at the completion of an in vitro permeability experiment. Examples of nonabsorbable compounds, which could be used as markers, are mentioned in Sect. 6.4.1.2. If tissue collection, storage, or isolation does not compromise the integrity of the membrane, marker compounds do not appear in the receptor chamber. Though passive diffusion is the primary mechanism for transport of most permeants across the buccal mucosa, carrier-mediated processes do play a role in the transport of nutrients and may play a role in the transport of some drugs. Also, the buccal mucosa is known to metabolize certain molecules. Therefore, in order to account for metabolic biotransformation and the effect of carrier-mediated processes, it is important to maintain tissue viability during the course of in vitro buccal permeation studies.

This viability of the tissue can be assessed using biochemical markers, microscopic methods, and the linearity of transport data [12]. Information regarding the metabolic activity of the tissue may be obtained by biochemical means, such as estimating adenosine triphosphate (ATP) levels and conducting glucose utilization studies, but these may not necessarily correlate with the preservation of barrier properties[63]. Other researchers have suggested that the tissue can be considered viable if there is no alteration in drug permeability over the duration of the in vitro experiment [88–90]. The viability of the tissue must be given careful consider-

ation, especially while studying the absorption of molecules that use specialized transport mechanisms. The MTT cell proliferation assay and histological evaluation can be used to assess the viability of tissues. Apart from the above-mentioned disadvantages, the tissue isolation procedure is time-consuming and most in vitro models have a limited potential for assay automation. Despite these disadvantages, in vitro models are helpful to compare the permeability of a series of chemically related compounds, or different drugs with the same pharmacological effect. The permeability of compounds under the influence of permeation enhancers may also be assessed.

These in vitro methods are easier to perform than in vivo studies and are conducted by researchers on a routine basis. Although several in vitro methods have been described in the literature, no single test is an accurate predictor of the in vivo performance of a dosage form. When performed under controlled conditions, they may be used for rank order correlations. If experiments are conducted using viable membranes that closely resemble the human buccal mucosa, the probability of correlation with in vivo studies increases. When favorable in vitro test results are not mimicked or predicted by similar results from in vivo studies, it is probable that the in vivo conditions were not fully understood, and matched, in the in vitro study.

6.5 Residence Time

Once the dosage form is in contact with the buccal mucosa, residence time often determines the extent of drug absorption. Residence time, in the simplest terms, is the duration of contact of a mucoadhesive dosage form with the buccal mucosa, to facilitate drug absorption. This could be evaluated by measuring the in vitro residence time, a test primarily designed for dosage forms that are meant to be retained at the site of application for extended periods. This method determines the retention time rather than measuring the force of adhesion. The simplest version of the method involves securing buccal formulations, e.g., buccal films, to the inside surface of a container, or to a glass plate, followed by the addition of medium to the container and the application of a gentle mechanical force. The force is applied by stirring the medium by moving the plate or by rotating the container itself until the film detaches or has completely eroded. In this case, no buccal membrane is required to conduct this study and the residence time data could provide basic information regarding the performance of the buccal dosage form.

A modification of this method involves the application of the buccal film to freshly isolated buccal membrane that has been secured to a glass plate. The whole assembly is then transferred to a vessel containing simulated saliva. Stirring is maintained in the medium and the time required for dislodging or erosion of the formulation gives the residence time[91]. Research groups have modified the above method to determine the residence time of buccal formulations using a model that is physiologically more relevant [92]. In this adaptation, the buccal tissue is fixed to

the inner side of the container and the buccal dosage form is moistened and fixed to the buccal mucosa by applying gentle force. The medium, which closely resembles that of chewing, or acid, stimulated human saliva (with respect to mucin content, pH, viscoelastic properties, and interfacial properties), is added to the container and the medium is stirred. The time required for the patch to detach from the buccal mucosa is recorded.

The in vitro residence time can also be measured using a modified disintegration test apparatus in which 800 mL of isotonic phosphate buffer (pH 6.75) maintained at 37 °C serves as the medium. The mucosal membrane, secured to the surface of a glass slab, is vertically attached to a side arm of the disintegration apparatus. One face of the buccal tablet/film is hydrated using isotonic phosphate buffer and the hydrated surface is brought into contact with the mucosal membrane. The glass slab is then allowed to move up and down such that the formulation remains completely immersed only at the lowest point. Residence time is the time required for complete detachment/erosion of the formulation from the mucosal surface [91].

In another method that estimates residence time in vivo, the placebo buccal dosage form is given to human volunteers. They were asked to moisten the dosage form and place it in the buccal cavity by the application of slight pressure. Volunteers refrained from eating and drinking during the test, and the time it takes for erosion or dislodging of the formulation is considered the residence time [46]. It should be noted that the in vitro residence time may not provide information about the strength of the mucoadhesive bond but it will be helpful to optimize formulations [93].

6.6 Dissolution Test

The selection of an appropriate drug product for a clinical study is often based on the vitro release profile [94, 95]. It is an important quality control tool and plays a key role in the research and development of drug products. A proper in vitro test should be able to provide information regarding the in vivo dissolution behavior of a drug product [96]. The dissolution test determines the rate and cumulative amount of drug released from the formulation. Oral transmucosal drug products are traditionally evaluated using a disintegration or dissolution apparatus. Sublingual tablets of ergoloidmesylate, ergotamine tartrate, and nitroglycerin, per the US Pharmacopeia (USP), are evaluated using a disintegration apparatus. This apparatus has a basket-rack assembly with a glass beaker (1000 mL) and the provision for upward and downward movement. Isosorbide dinitrate sublingual tablets are evaluated using USP apparatus II. The evaluation of buccal delivery systems involves the use of milder conditions when compared to conventional dissolution testing. Several buccal dosage forms are meant to release the drug from one side only. Therefore, modifications are made to ensure that the drug is released from only one face of the formulation. Water is the simplest dissolution medium that is used for dissolution testing of buccal dosage forms but the selection and quantity of dissolution media used differ widely.

USP dissolution apparatus II, at a paddle speed of 50 rpm with 900 mL water as dissolution medium has been specified to evaluate sublingual tablets of isosorbide dinitrate [97]. Buccal mucoadhesive tablets of hydrocortisone hemisuccinate were evaluated by Fabregas and Garcia [97] using the type III apparatus at a rate of 20 strokes/min. The paddle over disc method has been used to evaluate the release of the drug from buccal films [98]. In a different approach, the buccal formulations were secured to the shaft of the USP type I dissolution apparatus and drug release studies were conducted using isotonic phosphate buffer as the dissolution medium [99].

A typical official in vitro release method or its modifications, in general, utilizes a large volume of medium and, hence, may not truly reflect the unique environment in the oral cavity. Hence several researchers have developed models that mimic the low liquid environment of the oral cavity [100–103].

Containers with low volumes of dissolution media were used to evaluate drug release from buccal formulations [104]. Ikinci et al. used Franz diffusion cells to study the release of nicotine from buccal tablets with 22 mL of phosphate buffered saline serving as the dissolution medium [105]. These nicotine buccal tablets were partially sealed using paraffin wax to provide unidirectional release.

Release of ketorolac from buccal films was studied using a flow-through type system [106]. Mumtaz and Ch'ng employed another method in which the buccal tablet was attached to a chicken pouch membrane [100]. A similar method used a continuous flow-through filtration cell with a dip tube [107].

The US Food and Drug Administration (FDA) has adopted the use of one or other of the standard USP dissolution apparatus, modifying the volume of solution and specifying a smaller vessel or a different impeller as shown in the examples in Table 6.1[108].

Table 6.1 Dissolution tests specified by the FDA for selected oral transmucosal products

Drug name/dosage form	Vessel volume	Impeller	Impeller speed (rpm)	Volume of medium (mL)
Buprenorphine and Buprenorphine + Naloxone sublingual tablets	Conventional (900 mL)	Basket	100	500
Buprenorphine + Naloxone sublingual film	Paddle over disk	Paddle	100	900
Fentanyl citrate buccal tablet	Small volume apparatus	Paddle	100	100
Fentanyl citrate buccal film[a]	100 mL	Basket	100	100

Conditions specified are for the 1.2-mg film; interestingly for lower dose films, the same conditions are specified except that the volume of medium is 60 mL

FDA Food and Drug Administration

[a] Conditions in place of Conditions

6.7 Mucoadhesion Studies

The key step in the development of mucoadhesive buccal formulations is the selection of a mucoadhesive polymer. Mucoadhesion studies provide valuable information regarding the choice of polymer and aid in the design and development of mucoadhesive formulations. The tests to study mucoadhesion can be classified as direct and indirect methods [93]. Direct methods measure the amount of force that is needed to disrupt the adhesive bond between the membrane in question and the polymer. A modified Wilhelmy plate method is one of the earliest methods that can provide important information regarding the choice of a polymer for buccal films [109]. The texture analyzer is often used in the literature in regard to measuring the detachment force and work of adhesion, which can be of help to characterize mucoadhesive polymers. Examples of other direct methods include a modified balance, a tensiometer, and atomic force microscopy. Indirect measurements are based on polymer–mucin interactions and examples include rheology, ellipsometry, electrical conductance, colloidal gold staining, the flow channel method, lectin-binding inhibition, and the fluorescent probe method. The structural requirements needed for a polymer to exhibit mucoadhesive properties can be evaluated by the fluorescent probe method [99]. The lectin-binding inhibition method is based on the colorimetric evaluation of an avidin–biotin complex, which can be used to evaluate the binding potential of mucoadhesive polymers to buccal epithelial cells. The lectin, cancanavalian A, has affinity toward the sugar moieties present on the surface of buccal epithelial cells. When a mucoadhesive polymer binds with the epithelial cells, the binding of cancanavalian A is inhibited, and this can be quantified colorimetrically [100]. Apart from these, numerous other methods have been used by researchers to screen the potential of mucoadhesive polymers for use in oral mucosal delivery [93, 110].

It should be realized that unlike residence time, mucoadhesion measures the force required for detachment of the dosage form from the membrane, and not the time it takes for removal (or erosion). Researchers tried to correlate mucoadhesive force with residence time and found that it does not correlate in the ex vivo situation [99, 111]. In general, a high mucoadhesive force for a formulation may not result in a long residence time. This is because the former is a result of the flexibility of the polymer chain and the charge density; the latter is based on the dissolution of the polymer [99].

6.8 Buccal Cell Cultures

Although the buccal mucosa isolated from animals is the most common model to screen compounds, it has some disadvantages: a tedious isolation procedure, damage to the tissue due to mastication while the animal was alive, limited surface area, the difficulty in automation of the analytical process, and a significant variability

in permeation data among replicates. Therefore, large-scale experiments involving the testing of many formulations or compounds are extremely time-consuming and expensive. Consequently, many groups have explored cultures derived from buccal epithelial cells as models for investigating in vitro buccal permeation. The potential applications of buccal cell culture models include large-scale permeability screening of compounds, the investigation of transport mechanisms, and the study of potential biotransformation occurring in the epithelial cells.

Tavakoli-Saberi and Audus [61] developed a model using hamster pouch buccal epithelial (HPBE) cell primary cultures over membrane filters coated with collagen. These cultured cells have similar morphological characteristics to that of stratified epithelia; there were no significant differences in the specific activity of certain enzymes between cultured and excised cheek pouch epithelium. However, these cultured cells failed to completely differentiate and only the superficial layers displayed terminal differentiation. The permeability of these cultured layers to water, dextran, and fluorescein declined to a minimum by the third day and then gradually increased before stabilizing by the seventh day. These cultured HPBE cells were, therefore, ready to be used after day 7. Due to the lack of terminal differentiation, the cultured HPBE cells were comparable to the human buccal epithelium, which is also not keratinized, and therefore the cultured cells could be used as a surrogate for human buccal epithelium. The monolayers grown from epithelial cells of Madin-Darby canine kidney (MDCK) cells were also explored as a model for investigating the buccal absorption of molecules [112].

The TR146 cell line is another model developed to study the buccal absorption of compounds [65, 113–115]. These cells are harvested from human squamous buccal cell carcinoma [116], and following culturing, they develop as an epithelium that resembles human buccal mucosa [114]. However, due to the cancerous nature of the original cells, this model has a lower barrier function when compared to porcine or human buccal epithelium [117, 118]. The TR146 cell culture model has been used to investigate the metabolism and permeability of drugs such as leu-enkephalin, which is susceptible to buccal enzymes [65]. In order to overcome the disadvantage of the TR146 cell line, cultures have been derived from the buccal mucosa of healthy individuals. The cultured cells have remarkable similarities to the original buccal tissue with respect to morphology, composition, and barrier function [119].

EpiOral™, is a three-dimensional, multilayered, highly differentiated model of human oral epithelium derived from normal human keratinocytes [120]. This model resembles human oral mucosa in terms of structure, lipid content and protein expression. There is also good batch-to-batch reproducibility in tissue cultures in terms of their barrier functions. Identical permeability parameters for naltrexone hydrochloride were reported by Rao et al. [121] using these cultures and porcine buccal membrane. A good correlation was observed between fentanyl permeation across tissue cultures and bioavailability in humans [122, 123].

There are many advantages with the culture model such as excellent reproducibility, availability in large scale, and viability. However, there are certain limitations with this model. For example, the number of compounds examined in vitro are very limited [120] and, hence, more studies are required to validate this model.

The effect of nonaqueous formulations on cell viability and functional integrity of the culture need to be evaluated. Various parameters such as the number of differentiated cell layers, rate of culture growth, and lipid composition may influence the process of drug transport. Overall, this buccal epithelial cell line appears to have potential as a screening tool for investigating passive transport of molecules across the buccal mucosa.

6.9 Synthetic Surrogates

As a substitute for excised animal buccal mucosa, synthetic membranes offer certain advantages such as a uniform porous path, decreased experimental variations (due to structural homogeneity), and the ability to screen a large number of formulations. Synthetic membranes can be used to rank various formulations based on their permeation through the membrane. Despite these advantages, the lack of a stratified nonkeratinized epithelium is a serious limiting factor to the use of synthetic membranes. The physicochemical properties of the drug do not influence its permeation across synthetic membranes to the same extent as biological membranes. Furthermore, they cannot provide information regarding drug absorption pathways, biotransformations, and interactions within the buccal epithelium. The presence of carrier-mediated transport mechanisms and the influence of permeation enhancers are also not readily discernable.

To evaluate the release of nicotine from buccal films, Pongjanyakul and Suksri used a synthetic membrane in place of a biological membrane. They used a cellulose acetate membrane mounted over a Franz diffusion cell as the substitute [124]. Lala et al. [125] used a dialysis membrane (cutoff 12–14 kDa) mounted over a Franz diffusion cell to study the release of ketoprofen from buccal films. Adhikari et al. also used a dialysis membrane to investigate the release of atenolol from buccal patches[126]. Similarly, a semipermeable membrane was used to assess the release of propranolol from buccal patches made of ethylcellulose. Although a few such studies have been performed, there is no compelling evidence to justify the use of synthetic surrogates in place of biological membranes for in vitro studies.

6.10 Pharmacokinetic Studies

Buccal absorption tests and in vitro permeation studies may provide some insight into the ability of a drug to permeate across the oral mucosal membranes, and of the feasibility of developing formulations of test drugs. No matter how detailed the testing, the information obtained may not be comprehensive. This is due to the fact that only a few parameters are taken into account in the design of in vitro tests, while several additional factors may also influence the overall performance of the dosage form in vivo. As a consequence, numerous drugs may demonstrate excellent

potential based on in vitro experiments, but only a fraction of these successfully enter the market. Hence, in vivo studies are usually required for drug development and regulatory approval.

PK studies in animals provide additional, useful data which may influence the direction of human testing. Pharmacokinetic studies can more closely evaluate the various biopharmaceutical factors affecting the performance of the dosage form in vivo. Information obtained from these can be used to refine the formulation to be used in subsequent clinical trials. While PK studies are more expensive, they usually provide significant information regarding a formulation. While conducting a PK study, appropriate models and methods must be chosen based on the drug and the information from in vitro studies. A carefully designed in vivo study avoids the need for further studies. The choice of the animal model is important.

In recent times, the dog and pig animal models have been extensively used to conduct in vivo studies. However, PK studies in rabbits are not uncommon. Dali and coworkers used rabbit and human models to conduct PK studies for propranolol, verapamil, and captopril [127]. Iga and Ogawa conducted PK studies in dogs in which they reported increased bioavailability, along with extended absorption time, of nitroglycerin and isosorbide dinitrate sustained release buccal tablets following gingival administration [128]. Examples of other studies reported in the literature using animals are summarized in a review by Patel and coworkers [129].

6.11 Conclusion

Despite decades of research, the ideal animal model for evaluation of buccal drug delivery systems remains elusive. Further research is required to develop in vitro and in vivo models that more closely resemble viable human buccal mucosa and cheaper alternatives that permit experiments on a larger scale. The development of improved buccal cell cultures is one area that can be explored to find a better alternative to human buccal mucosa for in vitro testing.

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Chapter 7
Design and Development of Systemic Oral Mucosal Drug Delivery Systems

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7.1 Introduction

There has been high interest in delivering drugs across the oral mucosa for the past three decades. Due to the relatively poor permeability of the oral mucosa, a limited number of drugs possess the inherent physicochemical properties to allow them to cross the mucosa in clinically relevant amounts. The advantages of the oral cavity as a site for drug delivery have stimulated interest in research which has focused on increasing the drug candidate list. These studies have led to many insights into the use of permeation enhancers and mucoadhesives to enhance the usefulness of oral mucosal drug delivery systems. This chapter examines the research in these areas and how they have resulted in extending the clinical opportunities for the use of the oral mucosa for drug delivery. It must be remembered, however, that oral mucosal drug delivery must offer a definite therapeutic advantage for it to be useful, such as reducing the first-pass effect, or the faster attainment of clinically relevant blood levels.

7.2 Systemic Oral Mucosal Drug Absorption

The absorption of drugs from the oral cavity has been examined in many in vivo and in vitro models. Such studies have provided the fundamental scientific rationale for the development and optimization of products used in the oral cavity. The

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pioneering work in this area was conducted by Beckett and coworkers who demonstrated in human volunteers that drug loss from a solution swirled around the mouth was a result of the drugs absorption across the oral mucosa [1, 2].

More sophisticated approaches using specially designed cells which were attached to the surface of the oral mucosa of a variety of test animals [3–5] and human volunteers [4, 6, 7] have been described in the literature. These models have been comprehensively reviewed by Rathbone et al. [4, 5]. The advantage of the perfusion cells is that the donor phase, which is continually being flushed and recycled across the oral mucosa, is shielded from contact with the environment of the oral cavity. Drug concentration, surface area, pH, and donor phase composition can be modified and closely controlled, allowing the effect of these factors on drug absorption to be critically assessed.

Other approaches have used a surrogate for human oral mucosal tissue. Such studies have, usually, used porcine buccal tissue. This tissue with its nonkeratinized epithelium is a close match to its human counterpart and has been used extensively in oral mucosal permeability studies [8–10]. Şenel et al. [11] have demonstrated that the bovine buccal mucosa can also be used in ex vivo permeation studies.

Most recently, cultured epithelial cell lines have been employed as an in vitro model for studying drug transport, barrier properties, and metabolic influences of the oral mucosa. As an example of this approach, nonkeratized buccal cells (Epi-Oral™) plated in six-well plates are used. These can be purchased from MatTek Corporation [12]. Various types of diffusion cells have been used in these studies. These include continuous-flow perfusion chambers, side-by-side chambers (Ussing chambers and Grass–Sweetana cells) and vertical diffusion cells (Franz cells).

7.3 Chemically Assisted Oral Mucosal Drug Absorption

One of the major disadvantages associated with drug delivery from the oral cavity is the low flux of drug which results in low drug bioavailability. In an attempt to increase drug flux across oral mucosa, various chemicals have been coadministered with the drug. Indeed, this approach is essential in order to deliver a wider number of drugs across the oral mucosa. However, it is critical that these compounds are safe, nontoxic, nonirritating, and reversibly reduce the barrier potential of the oral mucosa [13].

A variety of different chemicals have been used as permeation enhancers across oral mucosal tissues. These include: bile salts such as sodium deoxycholate, sodium glycocholate, sodium taurocholate, and sodium glycodeoxycholate; fatty acids such as lauric acid, capric acid, and oleic acid; chelating agents such as sodium EDTA or salicylates; surfactants including sodium dodecyl sulfate, polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether, and benzalkonium chloride; and a miscellaneous group that includes cyclodextrins and Azone [14, 15].

More recently, chitosan and its derivatives have been shown to enhance permeation of large hydrophilic molecules across the oral mucosa [16].

The decrease of the barrier properties of the oral mucosa observed following the application of chemical enhancers arises as a result of one of several mechanisms.

These include: an increase in cell membrane fluidity; extraction of the structural intercellular and/or intracellular lipids from the membrane; the alteration of cellular proteins; or changing the mucus structure or rheology [13, 15]. Ultimately, the efficiency of the selected enhancer depends upon the physicochemical properties of the drug, the administration site, and the nature of the vehicle [17].

7.4 Electrically Assisted Oral Mucosal Drug Absorption

Recently, physical methods such as the application of an electric field or the process of sonophoresis have been used to increase the permeation of drugs across the oral mucosa. The application of an electric field provides an additional driving force on drug ions (iontophoresis), forces water (or bodily fluids) to flow together with the dissolved drug or metabolites, or temporarily modifies tissue structures to make them more permeable (electroporation) [17–19].

These result in the facilitation of drug permeation and increased amounts of drug to permeate the oral mucosa. However, in the oral mucosal drug delivery field, this approach is still in its infancy and many technical issues need to be overcome before it becomes an accepted method to improve the penetration of drugs. The phenomenon of iontophoresis has been known for many years and applied widely in transdermal drug delivery. In recent years, it has also been investigated for enhancement of drug transfer across buccal mucosa [20–24].

The buccal mucosa provides an attractive area for electrical drug delivery. In vitro experiments have shown that buccal mucosa at pH 7.4 behaves as a cation exchange membrane and nonlinear resistor. It was reported to have a lower resistance and to be more permeable to water than the skin [18]. The anatomy of the buccal mucosa allows location of the electrodes set on the same surface. Another possibility is to place the donor electrode inside the cheek and the acceptor electrode on the external side of the cheek. This opposite location is suggested to increase the current efficiency of drug transfer [18]. A miniature, computerized drug delivery system has been described and incorporated into a dental appliance [25, 26].

An intraoral electronic device (IntelliDrug) for the buccal delivery of naltrexone was developed [27, 28]. This device was shown to induce continuous, long-lasting, controlled levels of drugs in pigs. Drugs used to treat Alzheimer's-type dementia were also loaded into the IntelliDrug device, and the permeation of the drug across pig oral mucosa was shown to be increased by iontophoresis [29].

7.5 Mucoadhesives

Other than the low flux associated with oral mucosal drug permeation, a major limitation of the oral mucosal route of administration is the need for the oral mucosal drug delivery system to be retained at the site of absorption. To achieve this, excipients must be formulated into the delivery system to enhance the system's ability to

retain itself at the administration site. For mucosal sites such as those found in the oral cavity, this is generally achieved through the use of excipients such as bioadhesive polymers or mucoadhesives [30, 31] (see Chap. 4). Many classes of polymers have been investigated for their potential use as mucoadhesives. These include synthetic polymers such as hydroxypropyl methylcellulose, polyacrylic acid, cyanoacrylate, and polymethacrylate derivatives as well as naturally occurring polymers such as hyaluronic acid, chitosan, and its derivatives [32–37].

7.6 Clinical Applications for Oral Mucosal Drug Delivery

The techniques described above have increased the number of drugs that are able to permeate the oral mucosa in effective amounts and, thus, the number of drugs that are potentially available for the development of oral mucosal delivery systems. As such delivery systems come to market and become successful products, further product development and research is stimulated.

7.6.1 Clinical Opportunities and Applications

The clinical assessment of oral mucosal delivery systems presents some special problems, and additional factors must be taken into account. First, mucosal dosage forms have very specific, and often unique, methods of administration in contrast to conventional tablets and capsules which are simply swallowed. Two products for the same indication may also have different methods of administration. For example, fentanyl containing effervescent tablet, Fentora® must be placed in a very specific area of the buccal cavity, or under the tongue, and must not be sucked or chewed; fentanyl-containing lozenge, Actiq®, must be actively administered by applying the drug-containing lozenge on a handle to the buccal area over a very specific time (15 min), the handle must be twirled, and the patient must suck on the lozenge. The testosterone-containing tablet, Striant®, must be applied to a specific area (between the gum and lip) and, if the patient needs to drink, a straw should be used to suck up the liquid on the side of the mouth opposite to which the drug has been applied.

For such oral mucosal delivery systems, administration instructions can be complex and unusual to the patient, but it is crucial to follow the manufacturer's administration instructions in every detail to ensure optimal function of the delivery system. For studies comparing two novel delivery systems, it is imperative to instruct clinical staff adequately so that one, or the other, dosage form is not inadvertently disadvantaged. In large multicenter trials, this becomes even more difficult, especially with new delivery systems where clinical staff may not have had the opportunity to become familiar with the product. It follows that a comparative trial of two novel delivery systems, which have specific dosing instructions as illustrated in the above examples, cannot easily be conducted as a blinded study.

To conduct a blinded study of this type, the design would have to include the double dummy technique. In a randomized, comparative trial of product A and product B, two treatments have to be administered at each treatment period. A particular patient may take product A and placebo B in the first period, whereas this patient takes product B and placebo A in the second period. In a placebo-controlled comparative trial of products A and B, the patient under consideration would take placebo A and placebo B in the third period. In this way, the study can be blinded to both the patient and the clinic staff. However, this adds a nontrivial layer of complexity to the preparation of clinical trial material, to the control of clinical supplies at the clinical sites, and to the reconciliation of clinical supplies.

Opportunities for the development of systemic buccal/sublingual delivery products are many and include:

a. Rapid delivery of drugs to treat conditions that need a quick pharmacological response
b. Delivery of drugs that cause side effects when delivered through the oral route (i.e., swallowed)
c. Drugs that are inactivated to a high degree by the harsh conditions of the gastrointestinal tract (GIT)
d. Drugs that undergo a high first-pass effect
e. Drugs that are presently administered only by injection

Pain and erectile dysfunction are examples of the first category. Fentanyl dosage forms, for the rapid relief of pain, were developed and the scientific and commercial success of Actiq and Fentora (Effentora) prompted the development of other fentanyl products for administration through the mucosa of the oral cavity.

The importance of metabolism of the drug during passage through the oral cavity mucosa is becoming increasingly understood (see Chap. 3) and this phenomenon should not be ignored. Nevertheless, the extent of metabolism is usually less in the oral cavity than in other parts of the GIT and, therefore, the oral cavity may be considered to offer an advantage, over swallowed medication, in this regard.

Peptides and other large molecules are usually administered by injection. Where such drugs are administered daily, or multiple times per day, the conversion of the drug to an oral transmucosal delivery system offers tremendous benefits to the patient who would self-administer the medication without experiencing pain. The development of large-molecule drugs for oral cavity administration has been slower to develop than small-molecule drugs for this route. The approval of an insulin product for oral mucosal delivery would spur the development of other biotechnology-derived large molecules. The launch of such a product in a large market such as the USA, Europe, or Japan would have a significant impact.

While even a cursory review of the literature reveals numerous examples of drugs that fall into the above categories (a–e), the development of effective dosage forms is not simple. In the first instance, the permeability of the drug through the mucosa of the oral cavity may be low. The next section discusses the reasons for the slow marketing approval of new oral mucosal dosage forms.

7.6.2 Why Are There Limited Oral Mucosal Drug Delivery Systems Available on the Market?

Despite the high interest in the oral cavity as a site for drug delivery, there are relatively few commercial products available for use by the patient [17] [38, 39]. There may be several reasons for this situation and these are summarized in Table 7.1. When added together, these individual reasons represent a huge challenge toward the design and development of an oral mucosal drug delivery system. Table 7.1 helps the reader understand the difference between the extent of research activity and the number of oral mucosal drug delivery systems actually reaching the market.

Some examples of products that have been launched, and of clinical applications, are described in the following sections.

Table 7.1 Reasons for the limited number of marketed oral mucosal drug delivery systems

Reason	Comment
Low-dose drugs with inherently difficult physicochemical properties often need to be formulated into oral mucosal drug delivery systems	The achievement of the optimal formulation may be difficult for the drug in question because its physicochemical properties may present formulation difficulties. These could include content uniformity issues, difficulty in attaining very fast dissolution or, conversely, attaining steady, sustained release over a predetermined time
There needs to be a good understanding of the underlying anatomical and physiological factors that influence the permeability of the drug across the oral cavity membranes	The developer may underestimate the complexity of these questions until the delivery systems are tested in vivo
There is often a need to incorporate enhancement strategies into the formulation in order to enhance the absorption of the drug	These must be safe, nontoxic, and not cause undue damage to the oral mucosa
The taste of the drug may become apparent if the drug is allowed to move around the mouth and come in contact with the tongue	This aspect may adversely affect patient acceptability
While an increase in the absorption rate and an enhancement of bioavailability are both desirable attributes, the extent of improvement from in vitro studies may have been overestimated during early development	When the product is tested in the animal, dose titration studies needed for product optimization or for registration purposes may prove to be difficult
With a novel route of administration, it may be more difficult convincing regulatory agencies of the acceptability of a new product	The agency may display greater circumspection, in keeping with their aim of protecting the public
Oral mucosal drug delivery research and delivery system development are often undertaken by smaller companies who do not have the resources of the larger pharmaceutical companies	The cost of researching and developing an oral mucosal drug delivery system should not be underestimated

7.6.3 Breakthrough Cancer Pain

Breakthrough cancer is a condition involving a transient exacerbation of pain that occurs either spontaneously, or in relation to a specific predictable or unpredictable trigger, despite relatively stable and adequately controlled background pain [40] .

Breakthrough pain may be a result of several causes including a direct or indirect effect of the cancer, the effect of the anticancer treatment, or the result of a concomitant illness [40]. It may result in a number of physical or psychological problems, and social complications. As a result, the presence of breakthrough pain can have a negative impact on the quality of life of the patient [41, 42]

Several products that contain fentanyl citrate, which is a potent fast-acting opioid agent, are available for application to the oral cavity (see Chap. 8). The bioavailability of oral mucosal fentanyl varies considerably by product type.

An early study, during the development of effervescent buccal tablet, Fentora, revealed high plasma levels of fentanyl at early time points, and a higher C_{max} (0.6412 versus 0.4073 ng/mL) and shorter median T_{max} (0.5 versus 2 h) for this product compared to the lozenge, Actiq [43] . In an open label study comparing 100, 200, 400, and 800 μg Fentora in healthy adult volunteers, it was found that there was a high early systemic exposure to fentanyl (illustrating the dosage form's utility in breakthrough cancer pain) and also that there was dose proportionality in the C_{max} and area under the curve (AUC) [44] .

The more widespread use of opioids for the treatment of noncancer chronic pain, in recent years, has revealed a greater incidence of breakthrough pain in these patients than was previously considered to be the case. Hence, there was a need to test breakthrough pain medications for their efficacy and side-effect profile when used for this indication. While Fentora was initially developed to treat breakthrough pain in cancer patients, it was potentially useful for this indication as well. Therefore, it was tested for its long-term safety and tolerability in treating breakthrough pain in patients who experienced chronic noncancer pain [45]. In this 18-month study involving 646 opioid-tolerant patients, most adverse events were mild to moderate in intensity and typical of opioid use. Most of the serious side effects were considered to be unrelated or unlikely to be related to the dosage form under test. Most patients reported improvement in their ability to function.

7.6.4 Nausea and Vomiting

Vomiting or throwing up is forcing the contents of the stomach up through the esophagus and out of the mouth. Nausea is the feeling of having an urge to vomit. Nausea and vomiting are common symptoms that can be caused by a wide variety of conditions. Several products are available for use on the oral mucosa. These include Buccastem and Emezine both of which contain prochlorperazine. Buccastem is a tablet containing 3.0 mg prochlorperazine maleate BP that is placed in the buccal area. It releases the drug over a few hours.

Domperidone (Motilium®) has long been used orally for the treatment of nausea and vomiting in adults and children. A recent, interesting paper describes the development of a domperidone-containing buccal film by hot melt extrusion [46]. In a pharmacokinetic (PK) study using 12 adult male subjects, the buccal film demonstrated a higher C_{max} (129.7 compared to 94.22 ng/mL) and AUC (455.1 compared to 304.7 ng.h/mL) than the oral (swallowed) dosage form. This is a reflection of the fact that the drug from the swallowed tablet undergoes significant first-pass metabolism. Unexpectedly, the T_{max} was slightly longer than that of the tablet (1.62 versus 1.5 h). The authors attributed this to the possibility that the film acted as a matrix delivery system.

7.6.5 Status Epilepticus and Serious Tonic–Clonic Seizures

Status epilepticus is a life-threatening condition in which the brain is in a state of persistent seizure lasting longer than 5 min, or recurrent seizures that occur for longer than 5 min, without regaining consciousness between seizures. Treatment is generally started after the seizure has lasted 5 min. Tonic–clonic seizures (formerly known as grand mal seizures) are a type of generalized seizure that affects the entire brain. Tonic–clonic seizures are the seizure type most commonly associated with epilepsy and seizures in general.

Buccolam® is an oral mucosal solution, containing 10 mg midazolam hydrochloride in prefilled oral syringes of 2.5, 5, 7.5, and 10 mg. Epistatus® is a liquid buccal formulation of midazolam maleate (10 mg/mL) that is available for the treatment of status epilepticus and serious tonic–clonic seizures in community settings. This is an unlicensed product, available as a "special" in the UK. These oral mucosal preparations provide a more convenient-to-use, and less embarrassing, option for a child compared to the competitor product, a rectal formulation of diazepam [47].

A head-to-head comparison of buccal midazolam and rectal diazepam was performed at a residential institution for adults with difficult-to-treat epilepsy [48]. The doses were titrated to the needs of the individual patient. In the course of this study, 80 episodes of seizures were treated and it was demonstrated that the seizures terminated faster (2.8 versus 5.0 min mean time) with buccal midazolam compared to rectal diazepam. All of the nursing staff (who administered the medication) and most of the patients who had both medications preferred the buccal midazolam which was easy to handle and socially more acceptable. The nursing staff felt that there was less potential for allegations of sexual abuse when administering the midazolam formulation.

7.6.6 Type 2 Diabetes and Obesity

Type 2 diabetes is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. The classic

symptoms are excess thirst, frequent urination, and constant hunger. Type 2 diabetes makes up about 90% of cases of diabetes. Obesity is thought to be the primary cause of type 2 diabetes in people who are genetically predisposed to the disease.

Oral antidiabetic medications are available to treat type 2 diabetes, e.g., the biguanides, the best known of which is metformin. Oral metformin is given in large daily doses (often 1000 mg/day) and this can cause gastrointestinal irritation and bloating. Oral medications do not always provide adequate control of the condition and, sometimes, the patient is required to self-administer insulin by subcutaneous injection. The injection causes pain, and there is the possibility of infection at the injection site. In addition, some patients have a fear of the needle and experience anxiety several times a day since injections are usually taken multiple times per day. Hence, alternate dosage forms representing more convenient methods of administration are being actively sought by researchers. A chewing gum [49] and oral mucosal liquid formulation [50] have been developed to treat this condition. The metformin-containing medicinal chewing gum contains a relatively smaller dose that is expected to reduce the gastrointestinal irritation and bloating caused by metformin.

The liquid formulation (Ora-Lyn) contains regular recombinant human insulin that is delivered to the buccal mucosa using the RapidMist device. This device provides a fine mist of formulation to the mouth. The delivered dose results in the formation of a thin membrane on the oral cavity mucosa. It consists of mixed micelles made from the combination of insulin and absorption enhancers that encapsulate and protect the insulin molecules.

The manufacturers of Ora-Lyn, Generex Biotechnology, studied the effectiveness of this spray in: (1) type 1 diabetic patients requiring insulin injections, (2) type 2 diabetics who were not adequately controlled on oral antidiabetic (Metformin), and (3) type 2 diabetics who were not adequately controlled with diet and exercise alone [51]. In the case of type 1 diabetics, when the treatments were administered 15 min after a standard meal, 100 units of insulin administered as Ora-Lyn lowered the blood glucose levels to a clinically relevant degree, though the effect was less than that of 10 units of insulin administered by subcutaneous (sc) injection. It was suggested that the convenience of the Ora-Lyn dosing would lead to greater compliance and, thereby, better control of the diabetic condition. In the second study, the addition of Ora-Lyn to metformin greatly reduced blood sugar levels after a standard meal. The postprandial glucose C_{max} as well as the AUC were significantly reduced with the addition of Ora-Lyn to the regimen. In the third study that was described, the addition of Ora-Lyn before a standard breakfast, reduced glucose levels significantly when compared to placebo. This suggested the utility of the addition of Ora-Lyn when diet and exercise were not sufficient in reducing obesity and elevated blood glucose levels. It is suggested by the authors that the introduction of Ora-Lyn, rather than oral hypoglycemics, such as sulfonylureas, early in the progression of the disease would help to preserve the functioning of pancreatic β cells, and will reduce the complications of type 2 diabetes. In all three studies, the tolerability of Ora-Lyn was reported to be good with no major episodes of hyper- or

hypoglycemia. There was no local irritation and the patients reported the device to be easy to use.

Recently, a phase 3 clinical trial was conducted at 14 sites in India by the Indian licensee of Generex , Shreya Life Sciences Pvt. Ltd. to assess efficacy and safety of Ora-Lyn versus Rapid Insulin, s.c. on subjects with type II diabetes who are inadequately controlled while on oral antidiabetic agents [52]. The hemoglobin A1c (HbA1c) was measured at baseline, 6 and 12 weeks. Oral-lyn™ was reported to reduce HbA1c more rapidly and was as effective as subcutaneously injected regular insulin at the trial's conclusion establishing noninferiority. Adverse events were rare and comparable between groups. It was shown to be easily used and well tolerated by patients.

7.6.7 Middle-of-the-Night Insomnia

Middle-of-the-night insomnia is characterized by difficulty returning to sleep after awakening either in the middle of the night, or too early in the morning. This type of insomnia is different from initial, or sleep-onset, insomnia which is a difficulty falling asleep at the beginning of sleep. Naturally, due to the disrupted sleep patterns caused by middle-of-the-night insomnia, the symptoms of the condition are fatigue and an inability to concentrate which, in the case of some tasks, such as driving, may prove to be dangerous. Intermezzo® is a sublingual tablet containing zolpidem tartrate which is available in two strengths (3.5 and 1.75 mg) for the indication of middle-of-the-night insomnia [53, 54]. Zolpidem is a well-known drug that has, traditionally, been used for insomnia at a dose of 10 mg. An extended release tablet formulation (Ambien CR®) containing 6.25 and 12.5 mg of the drug is also available. The lower dose product for middle-of-the-night insomnia (Intermezzo) dissolves fairly rapidly and a large portion of the dose is absorbed sublingually for a rapid effect. In a study comparing the 3.5 mg sublingual formulation with the 10 mg immediate release (IR) swallowed tablet, it was demonstrated that the mean plasma concentration at 15 min and the AUC from 0 to 15 min was higher for the sublingual formulation despite the much higher dose of the IR formulation [55]. This illustrates the higher absorption rate for the sublingual formulation which is expected to translate to more rapid sleep onset.

7.6.8 Oral Chronic Graft-Versus-Host Disease

Oral chronic graft-versus-host disease (GVHD) may occur after a bone marrow or stem cell transplant in which a patient receives a donor's bone marrow tissue or stem cells (allogeneic transplant). The transplanted cells regard the recipient's body as foreign and attack the recipient's tissues. Thus, this condition resembles an autoimmune disorder. Topical application of budesonide to the oral cavity has been

suggested as an add-on therapy for this condition [56]. The peroral bioavailability of budesonide is low due to extensive first-pass metabolism by the CYP 3A system. Since the bioavailability of budesonide via the buccal route was not known with respect to normal subjects or GVHD patients, a study was conducted to determine the PK parameters [57]. A concern, which prompted the study, was the potential for budenoside to be well absorbed through the oral mucosal tissues, but not metabolized to the same extent as it is during passage through the small intestine and liver. This could lead to potentially toxic levels. In healthy subjects, buccal administration resulted in lower systemic exposure to budenoside, compared to peroral administration. The relative bioavailability was 18–36% compared to oral administration. However, in GVHD patients the relative bioavailability was approximately 100%. There were significant differences in the C_{max} (0.18 versus 0.77 ng/mL) and AUC (1.14 versus 4.37 ng.h/mL) of healthy subjects compared to patients. The similar elimination half-lives of budesonide in both groups indicates that impaired systemic metabolism was not the reason for the higher blood levels. Rather, it is the increased uptake by impaired mucosa that is the major factor.

While oral lesions (e.g., induced by chemotherapy) are known to increase buccal absorption (and their presence is usually an exclusion criterion in clinical studies), this work demonstrates that other disease conditions may also alter buccal absorption.

7.6.9 Muscle Spasticity

Spasticity is defined as velocity-dependent resistance to stretch, where a lack of inhibition results in excessive contraction of the muscles, ultimately leading to hyperflexia (overly flexed joints). It mostly occurs in disorders of the central nervous system (CNS) impacting the upper motor neuron in the form of a lesion, such as spastic diplegia, but it can also present in various types of multiple sclerosis, where it occurs as a symptom of the progressively worsening attacks on myelin sheaths and is thus unrelated to the types of spasticity present in neuromuscular cerebral palsy-rooted spasticity disorders. An oral mucosal preparation called Sativex® has been developed to relieve this condition in multiple sclerosis patients suffering from muscle spasticity [58]. It is an oral mucosal spray containing delta-9-tetrahydrocannabinol and cannabidiol. The spray should be directed at different sites on the oromucosal surface, changing the application site each time the product is used. This product has been shown to reduce neuropathic pain [59].

7.6.10 Hypogonadism

Male hypogonadism is a condition in which the testes do not produce enough testosterone, the hormone that plays a key role in masculine growth and development

during puberty. Striant® is a buccal system that provides a novel treatment option for men who require testosterone replacement therapy for a deficiency or absence of endogenous testosterone associated with hypogonadism [60]. Insertion of Striant® twice a day, in the morning and in the evening, provides continuous systemic delivery of testosterone, thereby producing circulating testosterone concentrations in hypogonadal males that approximate physiologic levels seen in healthy young men (300–1050 ng/dL). Following the initial application of Striant®, the serum testosterone concentration rises to a maximum within 10–12 h.

7.6.11 Opioid Dependence

Opioid dependence is a medical diagnosis characterized by an individual's inability to stop using opioids (morphine, heroin, codeine, oxycodone, hydrocodone, etc.) even when objectively it is in his or her best interest to do so. The characteristics of people with opioid dependence are preoccupation with a desire to obtain and take the drug and persistent drug-seeking behavior. Several oral mucosal tablet preparations have been developed to treat this condition. Subutex® and Suboxone® are tablet formulations containing buprenorphine for initial treatment of opioid dependence. Subutex® is available as 2 mg sublingual tablets containing buprenorphine hydrochloride. The tablet usually fully dissolves under the tongue within 5–10 min. Suboxone® is a sublingual tablet containing 8 mg buprenorphine hydrochloride and 2 mg naloxone hydrochloride dehydrate, or 2 mg buprenorphine hydrochloride and 0.5 mg naloxone hydrochloride dihydrate. This formulation was intended for home use and the naloxone was included to deter extraction from the tablet and intravenous administration.

In 2010, Suboxone® sublingual film was developed using Monosol Rx's PharmFilm technology. The film was claimed to be preferred by patients due to the fact that it dissolved faster and had a better taste [61]. In 2012, the company withdrew Suboxone tablets from the US market, citing cases of accidental paediatric consumption as the reason. The fact that the films strips were individually wrapped was a safeguard against accidental consumption [61].

In a study of 92 patients who had been treated with buprenorphine/ naloxone tablets, a double-dummy technique was used to randomize patients to either the tablets (no change in therapy) or the film dosage form [62]. Doses were not changed from that at which the patients had previously been stabilized, using the tablet formulation. This was a parallel group trial over 31 days. The study demonstrated dose equivalency of the two dosage forms, and clinical outcomes were comparable between the tablet and the film groups. No significant differences were noted between the two groups with respect to trough buprenorphine or norbuprenorphine concentrations, adverse events or treatment outcomes. The film was faster to dissolve than the tablet (173 versus 242 s) and patients preferred the film.

7.7 Concluding Remarks

To date, several new oral mucosal drug delivery products are available for clinical use and more are expected to appear in the market in the near future. Indeed, the oral cavity is likely to be one of the routes of drug delivery that becomes important in the future. Great challenges face formulators who aim to systemically deliver drugs across the oral mucosa; however, their innovative solutions to these challenges will provide drug delivery systems that include ingredients that manipulate the bioavailability of drugs across the oral mucosa and provide a convenient, patient-acceptable means to relieve clinical symptoms.

Given the current state of the pharmaceutical arena, the oral mucosal route of administration is ideally suited to improve the delivery of several existing drugs. The developed delivery systems would need to offer market differentiation for these drugs through enhanced, innovative, nonpainful, and patient friendly delivery systems which, if optimally developed, will offer a definite therapeutic improvement. We complete this chapter by presenting Table 7.2 which summarizes the clinical studies that have been performed using buccal and sublingual delivery systems in the past 5 years.

Table 7.2 Clinical studies performed using buccal and sublingual delivery systems in the past 5 years

Drug	Delivery system/ application	Treatment	Subjects	Results/reference
Budensonide (synthetic glucocorticosteroid)	Effervescent buccal tablet containing 3 mg budesonide used to prepare mouthwash *Oral rinsing Buccal*	Add-on therapy for oral cGVHD	Healthy volunteers Patients with oral cGVRD	2% of a buccal dose of budesonide achieves systemic circulation in healthy individuals; that fraction is 10% in patients with oral cGVHD, probably because of alterations in drug uptake and metabolization. [57]
Naltrexone (opiod antagonist)	Electronically controlled intraoral device (IntelliDrug device) *Buccal*	Treatment of opiate addiction, alcoholism, and smoking cessation	Healthy volunteers	The transbuccal route resulted in efficiency 4–17 times higher than conventional per os route. [28]
Asenapine (psychopharmacologic agent)	Fast dissolving tablet *Buccal Sublingual Supralingual*	Treatment of schizophrenia in adults with bipolar I disorder with or without psychotic features in adults	Healthy male volunteers	Absorption was increased with buccal administration versus the recommended sublingual route The tolerability and safety was not adversely affected by variability in the intraoral placement of the fast dissolving tablet [63]

Table 7.2 (continued)

Drug	Delivery system/ application	Treatment	Subjects	Results/reference
Nicotine	Patch[a] *Buccal*	Nicotine Replacement Therapy (NRT)	Smokers aged 18 years and over and reporting smoking ± 10 cigarettes per day for at least 5 years	In smokers with medical comorbidities and highly motivated to quit, adaptation of the nicotine replacement therapy daily dose according to saliva cotinine does not appear to be substantially superior to standard nicotine replacement therapy use [68]
Midazolam (fast-acting benzodiazepine)	Solution (Epistatus, Dales Pharmaceuticals) *Buccal*	Treatment of acute repetitive seizures	Adult residential patients with severe epilepsy	Buccal midazolam at least as effective as rectal diazepam with little or no side effects, and administration easy to handle and socially more acceptable than the rectal route [48]
Fentanyl (opioid receptor agonist)	Mucoadhesive tablet *Buccal*	Treatment of breakthrough pain	Men and nonpregnant women between 18 and 80 years of age who had a 3-month history of chronic pain	Fentanyl buccal tablet resulted in more rapid onset of analgesia and was generally well tolerated in comparison with oxycodone for the treatment of BTP in opioid-tolerant patients [65]
			Thermally induced hyperalgesia pain model in healthy volunteers	Based on these measurements, FBT was ~ 45-fold more potent than intravenous morphine 60 min after administration Caution should be used before applying these results to chronic pain patients, who often have numerous comorbidities and concomitant medications [66]
	Soluble film *Buccal*		Healthy subjects	Peak fentanyl plasma concentrations and overall exposure increase in a dose-proportional manner [67]

Table 7.2 (continued)

Drug	Delivery system/ application	Treatment	Subjects	Results/reference
Insulin	Spray *Buccal*	Treatment of type-2 diabetes	Type 2 patients between 18 and 75 years of age with at least 1 year of use of oral antidiabetes medications	Significantly lowered the Hemoglobin A1c at 6 and 12 weeks compared to baseline [50]
Domperidone	Hot-melt extruded (HME) films *Buccal*	Treatment of motion sickness	Healthy male volunteers	Bioavailability from the optimized buccal films was 1.5 times higher than the oral dosage form [47]
Miconazole	Mucoadhesive tablet Troch *Buccal*	Treatment of oropharyngeal candidiasis (OPC)	Patients confirmed as HIV positive, and with confirmed evidence of OPC	Once-daily buccal tablet noninferior to tronch five times daily in the treatment of OPC in HIV positive patients Buccal tablet offers an effective, safe, and well-tolerated topical treatment [68]
9-δ-tetrahydro-cannabinol and cannabidiol (THC/CBD) (1:1 ratio)	Spray *Buccal*	Add-on therapy for moderate-to-severe multiple sclerosis (MS) treatment-resistant spasticity symptoms	MS patients	Relief of MS-related spasticity in the majority of patients who were previously resistant to treatment Clear improvements in MS spasticity-associated symptoms, activities of daily living, and quality of life [58]
		Adjuvant therapy for treatment of chronic pain in patients with advanced cancer	Patients with cancer-related pain experiencing inadequate analgesia despite chronic opioid dosing	The long-term use was generally well tolerated, with no evidence of a loss of effect for the relief of cancer-related pain with long-term use [59]

Table 7.2 (continued)

Drug	Delivery system/ application	Treatment	Subjects	Results/reference
Testosterone	Tablet *Buccal*	Hypogonadal men	Testosterone-replacement therapy(TRT) for male hypogonadism	Testosterone is released in a manner similar to the normal daily rhythm of endogenous testosterone secretion; well tolerated, with gum-related disorders such as irritation, inflammation, and gingivitis [60]

cGVHD chronic graft-versus-host disease, *HPMC* hydroxypropyl methylcellulose, *MC* methylcellulose
[a] Nicopatch® , Laboratoire Pierre Fabre Santé, Boulogne, France

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Chapter 8
Tablets and Other Solid Dosage Forms for Systemic Oral Mucosal Drug Delivery

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8.1 Introduction

Conventional tablets delivered through the gastrointestinal (GI) tract present a number of challenges to the effective and rapid delivery of the active pharmaceutical ingredient (API) such as first-pass effect in the liver, food effect on absorption, solubility, and metabolism in the intestinal or gastric fluids, delayed absorption, among others. To mitigate these issues, formulation scientists have explored various dosage forms that target an alternate route for drug delivery, such as ocular, topical, suppositorial, mucosal, or parenteral formulations. These routes seek to provide local delivery to the affected area or systemic delivery without the challenges in the oral route. One out of the delivery options that is particularly of interest when rapid onset is desired is based on exploiting the oral transmucosal (OTM) route. Pertinent to this route, various dosage forms are available such as lingual sprays, buccal patches, chewing gums, lozenges, lozenges on a stick, troches, sublingual tablets, and buccal tablets. The OTM route is advantageous because it is a noninvasive drug delivery method as compared to, for example, injection or implantation; the drug absorbed avoids first-pass metabolism in the GI tract and liver, and it provides a shorter onset time due to rapid absorption of the drug directly into the abundant blood vessels that line the oral cavity. OTM delivery is simple, has the potential to increase patient compliance, and can be administered by the patient or a caregiver with minimal discomfort.

This chapter will focus mainly on the formulation and characteristics of solid dosage forms used in transmucosal drug delivery, which comprise the majority of the dosage forms in this category, although the strategies and issues discussed

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herein will likely stay relevant for other dosage forms as well. The solid dosage forms most popular for transmucosal delivery include sublingual tablets, buccal tablets, buccal patches, and lozenges. These dosage forms generally fall into two categories: an "open" delivery system (such as lozenges and sublingual/buccal tablets) wherein the drug release is influenced by surrounding conditions such as saliva pH, rate of saliva secretion, and other factors outside of control of the formulation; and a "closed" system (such as buccal patches) wherein the environment inside the patch, i.e., the formulation, determines the release of the drug and allows the use of agents such as permeability enhancers to modify the performance which might otherwise be difficult. Solid dosage forms for OTM drug delivery generally consist of the active ingredient(s), filler(s), binder(s), disintegrant(s), flavor(s), and lubricant(s). They may also contain effervescence agent(s), pH modifier(s), sweetener(s), or other excipients depending upon the technology used. The solid dosage forms can be made by a variety of manufacturing methods such as freeze-drying, injection molding, or direct compression; although the latter is the most common and preferred route due to the large scale and economic cost of these operations. Excellent organoleptic properties of the formulation are also an important consideration as the tablet disintegrates in the mouth and patient preference is influenced by the taste or aftertaste left from the unit.

Any formulation approach for oral mucosal drug delivery would have to take into account a number of factors specific to this route, which may complicate the drug delivery. First, there is only a small amount of solvent (saliva) in which the tablet can dissolve; furthermore, the amount of saliva produced varies greatly depending upon the circumstances and between individuals. Generally, salivary glands produce on average between 800- and 1500-mL saliva each day. In the rested, unstimulated state, salivary glands produce approximately 0.5-mL saliva each minute, and in the stimulated state the number may increase to 1–3 mL/min. The residence time of OTM drug delivery systems is usually in the range of 15–30 min, wherein the total saliva produced is about 15–30 mL and is small compared to about 600–1000 mL of fluid that may be present in the GI tract. Whole saliva is a complex mixture of parotid, submandibular, sublingual, and minor salivary gland secretions mixed with leukocytes, bacteria, crevicular fluid, and sloughed epithelial cells [1]. Saliva has low molecular weight components, mainly electrolytes such as ions of bicarbonates, nitrate and nitrite, thiocyanate and hypothiocyanite, sodium, potassium, chloride, calcium, phosphate, and fluoride, in addition to glucose, ammonia, and urea. Saliva also has high molecular weight components such as proteins (enzymes such as α-amylase, lysozyme, peroxidase, esterases, proteases, among others, glycoproteins). The pH of saliva is usually above 6.0 which is maintained by a strong buffer capacity mainly by the carbonic/bicarbonate system, the phosphate system, and the proteins [2]. As a result, the pH of saliva varies according to the concentration of this buffering system. To evaluate the OTM delivery, the solubility of the drug in saliva and compatibility of the two need to be considered while taking into account saliva's salt and protein concentrations and the pH. Once the drug is dissolved, absorption across the oral mucosa can be described by Fick's first law of diffusion. This law states that the mass rate of drug absorbed is directly proportional

to (1) the difference in the drug concentration in the solution and in the blood, (2) the diffusion coefficient of the drug in the oral mucosal tissue, (3) the contact surface area of the dissolved drug and the oral membrane, and (4) partition coefficient of the drug between the solution and oral mucosal tissue; it is inversely proportional to the thickness of the oral membrane.

The 15–30-min time period over which the drug has to dissolve and then get absorbed is also much shorter than for conventional swallowed tablets wherein a tablet may remain in the GI for several hours. The contact surface area between the drug and the oral cavity is also small due to the localized nature of the delivery method and can directly determine drug absorption. This factor can be mitigated to a certain extent by increasing the contact time, by slowing the rate of drug dissolution and tablet disintegration. The surface area of the oral cavity is about 200 cm^2 and is extremely small compared to the surface area of other absorptive surfaces, e.g., GI tract (350,000 cm^2) and skin (20,000 cm^2) [3]. The smaller overall oral surface area and contact area with dosage form (especially if the tablet is held in place, such as with buccal tablets, and also depending on tablet size) reduce the exposure of the dissolved drug and probability for absorption. When the dosage unit dissolves, any drug solution that is not absorbed will be swallowed this will eliminate any further OTM absorption. As with the oral route, the size of the molecule being delivered also has a significant impact on the rate of absorption through the mucosal route. Generally, drugs that are absorbed well in the GI tract may not be well absorbed through the oral mucosa because the mucosa of the mouth is less permeable than the intestinal mucosa. Before the drug can be absorbed from solution, it has to partition into the oral membrane. The partition coefficient of the drug is a thermodynamic property of the molecule depending on its chemical structure; however, it also depends upon the two media involved, in this case the composition of the saliva and the composition of the oral mucosa. All of the above factors present significant challenges in drug delivery through the OTM route.

There are several strategies that formulation scientists utilize to overcome the challenges involved in drug delivery through this route. The solubility of the drug can be altered using pH modifiers, cosolvents, or surfactants, in the dosage unit depending upon whether the drug is ionizable or not. A different salt form may also need to be considered. By adding these excipients, the local environment can be altered to a more favorable composition. Changes to the formulation may however need to be carefully considered because adding these excipients may negatively impact other characteristics such as partition coefficient, rate of dissolution (which is kinetic property), and chemical stability. The rate of dissolution can be manipulated by the tablet characteristics (such as size, hardness, and porosity), selection of excipients (water soluble or not), and by addition of disintegrating agents. The permeation of the drug in the oral mucosa can be altered by using permeability enhancers such as bile salts. Effervescence agents, which include compounds that produce gas, have been added to formulations to serve the dual role of providing a dynamic pH variability and enhance drug penetration to maximize drug absorption. Examples of products based on this technology have been presented later in this

chapter. The mechanisms behind enhancing drug absorption via the effervescence agents are thought to be the following:

a. Reduce the thickness of the mucosal layer and/or viscosity
b. Increase the passive diffusivity across the membrane
c. Induce a change in the structure of the cells in the membrane
d. Increase the hydrophobicity of the cellular membrane and allow for higher drug partitioning

In the following portion of the chapter, examples have been discussed of commercial products and concepts in the development stage that elucidate the range of therapeutic areas and formulations that are amenable by the OTM route. The examples also include a direct comparison with other routes of administration and show where OTM delivery has been a successful method of delivery acknowledged by patients.

8.2 Nitroglycerin

Glyceryl trinitrate (nitroglycerin) and many organic nitrate esters are a popular choice for treating ischemic heart disease [4]. These molecules are potent venodilators and have arteriolar vasodilating ability, which helps patients with congestive heart failure by producing a reduction in the left ventricular filling pressure. In addition, pulmonary artery and right arterial pressures decrease after administration of nitrates and help patients with biventricular failure. Recently, intravenous (IV) delivery of nitroglycerin has also been of interest for acute myocardial infarction and for controlling blood pressure during surgeries or acute cardiovascular procedures. The exact mechanism of action of nitrates is not known, but it relaxes the vascular smooth muscle resulting in vasodilation of the veins, arteries, and arterioles. New nitrate delivery systems have recently been developed and are of much interest due to the dosing flexibility they offer. These systems include oral spray, sublingual tablets, buccal or transmucosal tablets, chewables, oral tablets, oral sustained release tablets, ointment, IV, and transdermal disk or patch.

In the 1960s and early 1970s, investigators and experts in cardiovascular diseases thought that long-acting oral nitrates were virtually useless [5]. Similarly, the Food and Drug Administration (FDA) rated nitrates as compounds of dubious effectiveness. However, the plethora of data now available no longer supports that view. A large body of data clearly indicates that orally administered nitrates are biologically active, clinically effective, and produce desired actions that last for several hours [5]. In addition, data on bioavailability of nitroglycerin and isosorbide dinitrate indicate that oral doses of both compounds produce therapeutic levels in plasma that are maintained for several hours [6, 7]. The peak plasma concentration and area under the curve (AUC) are associated with the dose. The absorption and plasma levels vary significantly from one individual to another which precludes the

use of standard doses. However, if sufficient oral nitroglycerin or isosorbide dinitrate is given, the aforementioned clinical effects are seen.

A number of studies have shown that the dosing regimen for nitrates has a profound effect on the appearance of tolerance, particularly in angina patients. It has been found that tolerance develops more rapidly when large doses, frequent dosing regimens, and/or long-acting formulations are utilized. To avoid tolerance, doctors should utilize the least amount of nitrates that achieves the desired clinical effect. Smaller doses, less frequent dosing intervals, and either short-acting compounds or longer acting compounds with interruptions for part of each 24-h period should be employed. Thus, the formulation of nitrates and mode of delivery requires careful consideration for maximum benefit.

The majority of organic nitrate esters used today in clinical medicine are compounds of nitroglycerin or isosorbide dinitrate. As discussed below, the OTM method is the preferred route for delivery of nitrates due to their fast absorption and onset.

8.2.1 Glyceryl Trinitrate Products

Sublingual Tablets Sublingual glyceryl trinitrate is considered the standard for treatment of acute angina attacks and remains the most widely prescribed cardiac drug in the world. The active is most commonly supplied in the tablet form with strengths of 0.3–0.6 mg with a dose up to 1.5 mg, as needed [8]. The active may produce a slight tingling or burning sensation when placed under the tongue. There is rapid onset of action, usually within 2–5 min of dissolution [9]. However, the time taken for dissolution can be variable and sometimes prolonged depending on the dryness of the mouth. Patients also need to be aware of various factors that may impact the stability of nitroglycerin tablets such as exposure to heat, light, moisture, improper packaging material, and keeping the same supply of tablets from an open bottle for longer than 12 weeks. The hemodynamic effects may last between 20 and 30 min. Sublingual nitroglycerin has also been shown to be effective in the management of retained placenta [10]. A sublingual spray form of glyceryl trinitrate has also become recently available. It is a stable lipid aerosol formulation of nitroglycerin with prolonged shelf life (3 years), which when sprayed directly on the tongue produces relief from anginal pain within 2 min and the effect lasts for up to 30 min. Each spray administers approximately 0.4 mg of nitroglycerin [9].

Buccal or Transmucosal Tablets Several years ago, an interesting formulation of these tablets was introduced in the USA, UK, and Germany consisting of glyceryl trinitrate dispersed in a methylcellulose matrix [5]. The tablet is placed in the buccal cavity between the upper teeth and the inner cheek wall. The cellulose matrix quickly forms a gel and holds the tablet in place for hours. Glyceryl trinitrate is rapidly released across the mucosal layers into the rich capillary bed of the mouth, which results in quick onset of action comparable with the sublingual formulation [5]. The therapeutic effect lasts for 4–6 h as long as the tablet is kept in place. Thus,

the buccal formulation provides both immediate and sustained action. This route of delivery has not been found to induce nitrate tolerance, possibly because of the rapid elimination of glyceryl trinitrate from the circulation once the tablet is fully dissolved. Bussman calculated the elimination half-life of nitroglycerin as 5–6 min with 75 % being eliminated within 10 min [9].

Buccal glyceryl trinitrate has been available in doses ranging from 1 to 3 mg. In subjects with heart failure, larger amounts have been used. Generally, subjects have been able to talk, drink, and eat with the tablet in place without difficulty for up to 6 h (on average, the duration was 4–5 h). Incidentally, one buccal formulation introduced in the USA as Susadrin® has not had much commercial success although it is understood to be an interesting and effective product [5].

Ryden and Schaffrath have shown that buccal nitroglycerin is an effective form of therapy for acute angina and for prophylaxis of stable angina [11]. Its pharmacokinetic profile is similar to IV nitrate and the drug has been shown to be a safe and well-tolerated alternative to IV isosorbide dinitrate for treatment of unstable angina.

Oral Glyceryl Trinitrate Tablets and capsules of glyceryl trinitrate in immediate or sustained release formulations have been available for many years. Although there are some experimental data that show the formulation with glyceryl trinitrate to be effective in patients with angina pectoris and congestive heart failure, the studies are less convincing than oral formulation with isosorbide dinitrate. In addition, oral glyceryl trinitrate is often used in insufficient dosage, e.g., 2.5–6 mg several times a day. However, it is recommended that treatment should be started with a minimum dose of 6.5–9 mg. Larger doses may be necessary for maintenance therapy.

8.2.2 Isosorbide Dinitrate

Oral isosorbide dinitrate is the most commonly prescribed long-acting nitrate in the world. The active is available in a number of other formulations: sublingual, chewable, IV, ointment, and transdermal.

Isosorbide dinitrate has a halftime of about 1–2 h and the effect lasts for 3–6 h. The active is metabolized into 2-mononitrate and 5-mononitrate both of which are bioactive. The isosorbide dinitrate is only 20–25 % bioavailable when taken orally. The usual dose is 5–80 mg [8].

Sublingual and Chewable Tablets Isosorbide dinitrate sublingual and chewable tablets are available for the treatment of angina attacks. In both formulations, the effect lasts for 1–3 h, which is much longer than with sublingual glyceryl trinitrate. But the onset of action of isosorbide dinitrate and its metabolites is slower than with sublingual glyceryl trinitrate.

Oral Swallow Tablets This is the second most popular choice in nitrate therapy other than sublingual glyceryl trinitrate. Oral isosorbide dinitrate is available in immediate release and sustained release formulations. A number of studies with

angina pectoris or congestive heart failure have shown this therapy to be effective. A concern for the development of tolerance exists and evidence shows that when administered four times a day, oral isosorbide dinitrate will reduce the duration of antianginal effect from 4 to 6 h to not more than 2 h. However, administration twice or thrice daily may avoid tolerance [12–14]. Oral isosorbide dinitrate has also been found to be effective against congestive heart failure over several months in many well-designed studies [15, 16].

8.2.3 Choosing the Right Delivery System

Nitrates are considered as initial therapy for most angina patients. Since a wide variety of formulations are available, it is important to consider the delivery route best suited to the needs of the clinical situation, while also taking into account patient preference. Specifically, the speed of onset of action and duration need to be carefully considered ([17], see Fig. 8.1). In acute attacks of angina, such as attack of angina pectoris, recurrent myocardial ischemia following myocardial infarction or episodes of chest pain, immediate nitrate action is required. In such cases, a rapid onset of nitrates is indicated. Formulations in this category include sublingual glyceryl trinitrate, and sublingual or chewable isosorbide dinitrate. Buccal or transmucosal glyceryl trinitrate have fast onset of action, comparable to sublingual glyceryl trinitrate, with the added advantage of sustained activity. Nitroglycerin oral spray is also effective and seems equivalent to the sublingual formulation [18]. In preventive care of angina episodes, several nitrate therapies should be considered, including oral glyceryl trinitrate or isosorbide dinitrate, buccal glyceryl trinitrate,

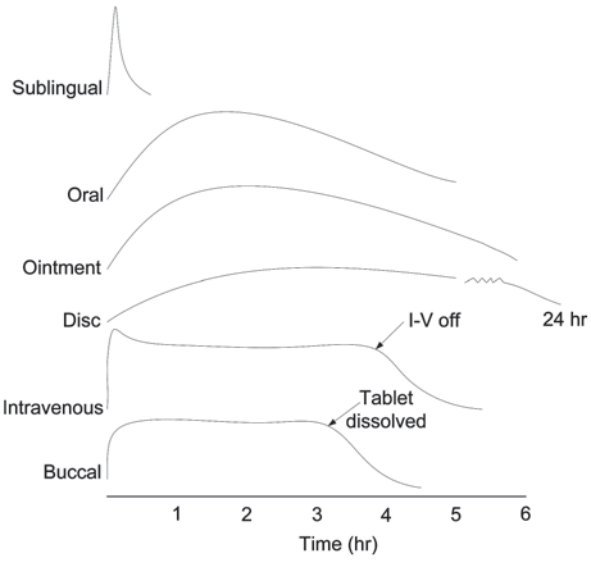

Fig. 8.1 Time course of onset of action and total duration of effect for the commonly used organic nitrate dosage forms. The pharmacokinetic profile for the oral spray appears to be identical to that of sublingual glyceryl trinitrate. Sustained formulations of oral glyceryl nitrate, isosorbide dinitrate, and isosorbide 5-mononitrate have a considerably longer duration of action than standard normal release preparations. (From Ref [17] with permission from Elsevier)

2% glyceryl trinitrate ointment, or the transdermal glyceryl trinitrate disk units. It should be noted that the ointment formulation may be impractical for many patients, although a new less leaking bandage unit of glyceryl trinitrate ointment makes it a more useful formulation for outpatient therapy. In hospitalized patients, the primary nitrate therapy includes glyceryl trinitrate ointment and IV glyceryl trinitrate. Oral glyceryl trinitrate or isosorbide dinitrate is also used, but it has a more variable time for onset and duration of action.

8.3 Sumatriptan Succinate

5-Hydroxytryptamine-1 receptor agonists (triptans) are the preferred first-line treatment for patients with no contraindications and have moderate to severe migraine [19]. Sumatriptan succinate is the first "triptan" drug which has been identified to have a substantial effect on the treatment of acute migraine and cluster headache. The drug is available in several dosage forms suitable for oral, intranasal, rectal, and subcutaneous administration. However, significant limitations in the delivery of sumatriptan remain, including the relatively poor oral bioavailability (about 14%) and the short half-life (2 h).

Recent impetus in the development of new formulations for this molecule was driven by the low oral and nasal bioavailability. Alternative drug delivery routes are of much interest given their advantages compared to traditional routes. Since migraine is commonly accompanied by nausea and vomiting, patients with these symptoms may not prefer the oral and nasal formulation due to low effectiveness and the bad taste of API (attributed to the 5-methanesulfonamide group). GI motility is reduced in migraineurs and absorption is delayed. Nasal administration may also not be suitable for patients with nasal congestion due to cold or allergies. In addition, for the nasal drug delivery, the absorption is limited by the mucociliary clearance system which is closely related to an individual's nasal morphology and physiology. All of the above factors limit the use of conventional, oral and nasal dosage forms. Currently, the subcutaneous form is the most effective therapy for patients with migraine and severe nausea or nocturnal crisis. However, the injection produces more adverse events in comparison to other triptans or other delivery forms. For instance, the injection site is slightly uncomfortable and potentiates events called "triptan sensation." Adverse events reported were highest for 6-mg subcutaneous sumatriptan (33% more adverse events than placebo), less with 100-mg oral sumatriptan (16% more adverse events than placebo) [21, 22], and with 50-mg oral sumatriptan (no more adverse events than placebo in one analysis [23], and 8% more adverse events than placebo in another analysis [24]). In consideration with the above discussion, new administration routes such as orally disintegrating tablets (ODT), buccal patches, lingual spray, among others have been developed to overcome the limitations associated with the delivery and efficacy of sumatriptan therapy. Compared with all other triptans, the administration of sumatriptan during the early phase of a migraine attack, while the pain is still mild and the allodynia is

not fully developed, increases the success rate of relieving the patient's pain [25]. For example, the administration of sumatriptan 100 mg during the aura phase of migraine can preempt the development of headache in 89% attacks [26]. The alternative routes of administration discussed here provide the possibility of rapid delivery of sumatriptan to aid in the pain relief.

Pharmacokinetics In oral administration, the optimum dose for sumatriptan is 50–100 mg, with no gain in efficacy at higher doses. Nasal administration is given at 10 or 20 mg, whereas subcutaneous formulation requires 6 mg every 24 h. The mean bioavailability is 96% with subcutaneous administration and decreases to approximately 14 and 25% with oral and nasal administration [27, 28]. The lower bioavailability in the oral and nasal route is due to the incomplete absorption followed with high first-pass metabolism. Oral bioavailability is species dependent (14, 23, 37, and 58% in humans, rabbits, rats, and dogs), which reflects the different efficiencies in first-pass metabolism [29]. Plasma half-lives of sumatriptan and its metabolites are approximately 1.7 and 2.3 h, respectively, after IV and oral administration [30]. Maximum plasma concentrations are achieved at median time of 10 min (range 5–20 min) after a single 6-mg subcutaneous dose, 1.5 h (range 0.5–4.5 h) after 100-mg oral dose, and between 1 and 1.75 h after 20-mg intranasal dose. The mean peak plasma concentrations of sumatriptan were 72 mcg/L after 6-mg subcutaneous administration, 77 mcg/L after 3-mg IV administration, 54 mcg/L after 100-mg oral administration, and 13.1–14.4 mcg/L after 20-mg intranasal administration [31].

8.3.1 Lingual Spray

In order to check the feasibility of increasing the absorption of sumatriptan from the oral cavity, a lingual spray formulation was developed [32]. Pharmacokinetic parameters, such as T_{max} and AUC have been evaluated for comparison with other delivery routes. With a 20-mg dose, the spray formulation showed fast transmucosal absorption (T_{max} 0.10–0.20 h) with a small fraction of dose delivered at low plasma concentration of 10 mcg/L of sumatriptan. Later, in GI absorption (T_{max} 2.0 h), there was a similar low plasma concentration of 10–12 mcg/L. The AUCs showed greater efficiency of systemic exposure with lingual spray as compared to the tablet formulation. The data show that absorption of sumatriptan across the oral mucosa is faster as compared to the tablets, and transmucosal delivery is a promising alternative [32].

8.3.2 Orally Disintegrating Tablet

The mean headache relief at 2 h for oral sumatriptan 100 mg, based on 20 placebo-controlled randomized clinical trials (RCTs), was reported as 59% and for the

placebo it was 28% [23, 33]. The mean headache relief at 2 h for oral sumatriptan 50 mg, based on six placebo-controlled RCTs was 59% and for the placebo it was 30% [23, 33]. The mean headache relief at 2 h for oral sumatriptan 25 mg, based on five placebo-controlled RCTs, was 56% and for the placebo it was 32% [23, 33]. One can conclude that the 100- and 50-mg doses have the same efficacy and are higher than the 25-mg dose. Both the 25- and 50-mg doses produce fewer adverse events than 100-mg sumatriptan. In one crossover trial [34], almost the same percentage of patients (35 and 31% for 100- and 50-mg doses, respectively) preferred the higher dose, as compared to only 21% for the 25-mg dose. Thus, some patients seem to prefer a more effective dose and are willing to tolerate the cost of more, transient, and often mild adverse events.

To increase the acceptance and effectiveness of oral sumatriptan, a new formulation has been developed to enhance tablet disintegration and drug dispersion, relative to conventional tablets. This fast ODT also helps alleviate the effects of gastric stasis that may accompany migraine. The ODT formulation of sumatriptan is bioequivalent to sumatriptan conventional tablets and is absorbed more quickly than conventional tablets (highest sumatriptan plasma levels were attained, on average, 10 min earlier (50 mg) and 15 min earlier (100 mg) compared with the conventional tablet) [35]. Two studies were conducted comparing the time to onset of relief from moderate to severe migraine pain with the ODT formulation of sumatriptan tablets 50 mg and 100 mg and placebo. Analysis of the pooled data showed that sumatriptan tablets provided significantly more pain relief than placebo as early as 20 min after dosing with the 100-mg dose and as early as 30 min after dosing with the 50-mg dose ($p \leq 0.05$). In the pooled data, the cumulative percentages of patients with pain relief by 2 h after dosing were 72% for the 100-mg dose and 67% for the 50-mg dose, as compared to 42% for the placebo ($p \leq 0.001$, both sumatriptan doses and placebo). The cumulative percentages of patients with a pain-free response by 2 h were 47% for the 100-mg dose, 40% for the 50-mg dose, as compared to 15% for the placebo ($p \leq 0.001$, both sumatriptan doses and placebo) [36].

More than half of the patients who were previously unsatisfied with lower doses of sumatriptan and less than very satisfied with their existing treatment regimen were more likely to be satisfied or very satisfied with sumatriptan ODT 100 mg [37]. This formulation allows for rapid and sustained restoration of functional ability in the acute treatment of migraine so that patients can quickly return to normal functioning at work and outside of work when administered early, when pain was mild for the acute treatment of a single migraine attack [38].

8.3.3 Buccal Patches

Shidhaye et al. [39] have developed a mucoadhesive bilayered patch with sumatriptan succinate using chitosan as the base matrix polymer with ingredients such as Povidone K30 and glycerin as pore formers. For the backing layer, ethyl cellulose was chosen due to its hydrophobicity, low water permeability, drug impermeability,

and moderate flexibility. The patch was designed to maximize buccal penetration of the drug with unidirectional release of the drug and greater surface area of contact. The unidirectional buccal route also allows the delivery of bitter drugs without the need for taste masking.

The patches were characterized with in vitro drug release studies, drug release from backing layer, in vitro bioadhesion, and in vitro residence time. The in vitro drug release studies showed that the drug release appeared to increase with an increasing amount of the hydrophilic polymer Povidone K30. This was expected since Povidone is known to absorb water and promote faster diffusion of the drug or dissolution of the membrane itself. To determine drug release from the backing layer, the bilayered buccal patch was placed between the donor and receptor compartment of a Franz diffusion cell. The donor compartment (exposed to the backing layer) was filled with simulated saliva at pH 6.8, while the receptor compartment (exposed to the active membrane) contained phosphate buffer of pH 7.4. Results showed that no drug was released in 120 min in the donor compartment of the diffusion cell. Ethyl cellulose was found to be impermeable to sumatriptan succinate and the patch was efficient with unidirectional release.

In vitro bioadhesion and residence time studies were done by attaching the patch on freshly cut porcine mucosa. The results showed that the concentration of chitosan had a more pronounced effect on the bioadhesion than the concentration of Povidone K30. At the same time, Povidone K30 had a negative effect on bioadhesion, i.e., when the concentration of Povidone K30 increased, the mucoadhesive strength decreased. The in vitro residence time results also indicated that the level of chitosan had a more significant effect than the level of Povidone K30. Patches that contained a low proportion of chitosan gelled fast and eroded rapidly. Again, Povidone K30 had a negative effect on in vitro residence time, i.e., as the concentration of Povidone K30 increased, in vitro residence time decreased.

Since sumatriptan succinate is a relatively hydrophilic molecule (log $P \sim 0.93$), it exhibits a low permeability through buccal mucosa, and therefore there is a need to enhance the buccal permeation with permeation enhancers that may cause perturbation and dissolution of paracellular fluid, increasing the paracellular transport. The authors explored three permeation enhancers, viz., transcutol, polysorbate 80, and dimethyl sulfoxide (DMSO) to improve penetration of sumatriptan succinate through the buccal mucosa. From the drug released in diffusion cell experiments, the permeability coefficients were calculated for formulations with the different permeation enhancers.

It was found that using 5 % transcutol and 1 % polysorbate 80 did not show much improvement in the permeation of sumatriptan succinate as compared to the effect of DMSO. DMSO increased the permeability of the drug rapidly, with maximum permeability at 3 % DMSO concentration in the formulation that was about 29 times the permeability coefficient of the formulation without any permeation enhancers. The formulation with the permeation enhancers was studied for adverse impact on bioadhesion, in vitro residence time, and drug release. However, no effect was seen on these characteristics, possibly due to the low levels of the permeation enhancers. The optimized formulation contained 3 % DMSO and had no significant effect on

the microscopic structure of the mucosa and thus appeared safe for buccal administration. The optimized formulation released 98% drug over 2 h with enhanced permeation without causing any tissue damage.

8.3.4 Buccal Tablets

A buccal tablet formulation has been described in the literature to enhance the bioavailability of sumatriptan succinate by avoiding first-pass metabolism [40]. The mucoadhesive buccal tablets were made using the polymers—Carbopol 934, hydroxypropyl methycellulose (HPMC) K4M, and HPMC K15M along with ethyl cellulose as an impermeable backing layer. Various formulations of the buccal tablets were made by changing the ratio of the polymers. The ex vivo mucoadhesive strength and mucoadhesion time was studied along with in vitro dissolution. Both the bioadhesive strength and mucoadhesive times were found to be satisfactory. Generally, an increase in the concentration of Carbopol showed an increase in mucoadhesion time, while increasing HPMC K4M and HPMC K15M showed a decrease in mucoadhesion time. In in vitro dissolution studies, the release of the drug from tablets was found to vary depending upon the type and ratio of matrix-forming polymers. The rate of drug release decreased with increasing concentration of HPMC K4M or K15M, possibly due to an increase in viscosity produced due to the gelling of these hydrophilic polymers. The release was non-Fickian with n value varying between 0.604 and 0.817. This indicates that both diffusion and chain relaxation were likely the prevalent mechanisms for drug transport.

In vivo bioavailability studies were also conducted on rabbits using an oral solution of sumatriptan succinate as standard. White male rabbits were made to fast for 24 h before drug administration and a bioadhesive tablet was fixed in the buccal position of the oral cavity. In oral administration, 10 mg doses of 25 mL aqueous solution were administered by a stomach tube. The mean plasma concentration of sumatriptan succinate with time following the administration of optimized formulation of buccal tablets and oral administration of solution is shown in Fig. 8.2.

With oral administration of sumatriptan succinate (10 mg) in solution form, the average maximum serum concentration of 482.20 ± 22.5 ng/mL was achieved after 2 h and the area under the serum concentration–time curve was 1200.90 ± 150.60 ng/mL. After administration of the optimized buccal formulation (T1), drug levels in serum were detectable until 12 h post dose with maximum serum concentration of 386.00 ± 15.80 ng/mL achieved 2 h after dosing and the area under the serum concentration–time curve was 1693.90 ± 91.50 ng/mL. The relative bioavailability of sumatriptan succinate following buccal administration was found to have increased to 140.78%, possibly due to the reduced first-pass metabolism when it is administered via the buccal route. It was shown that the buccal route may be a promising alternative to overcome the problems of poor and erratic oral bioavailability of sumatriptan succinate.

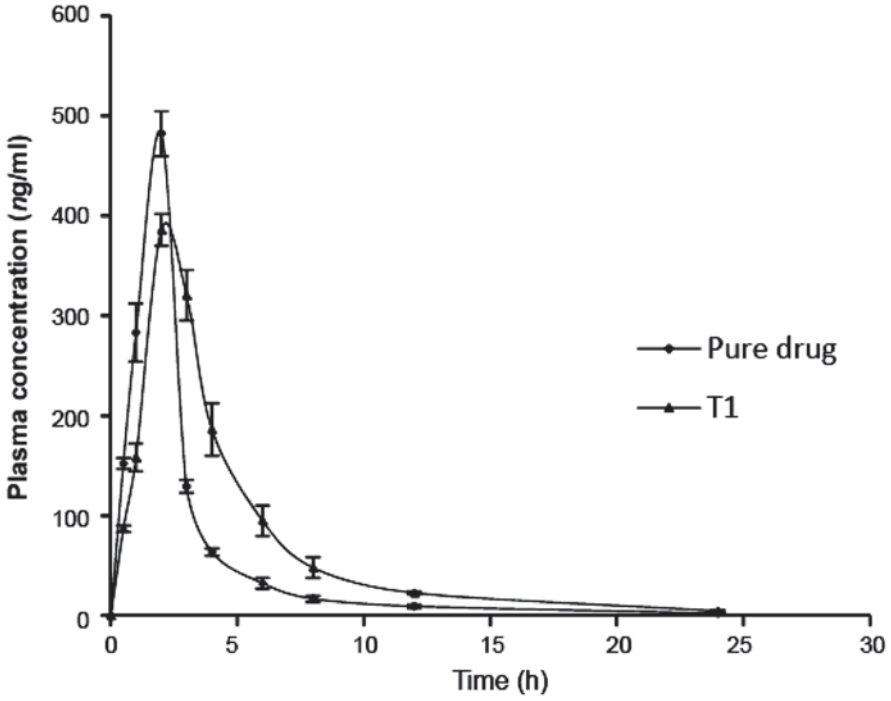

Fig. 8.2 Plasma concentration time profile of sumatriptan succinate after oral and buccal administration in rabbits. (From Ref [40], copyright of DARU Journal of Pharmaceutical Sciences)

8.4 Selegiline and Piroxicam

Selegiline is a selective monoamine oxidase-B (MAO-B) inhibitor that has been used for decades as an adjunctive therapy with Levodopa in the treatment of Parkinson's disease (PD) [41]. Several studies had suggested that oral selegiline is of moderate benefit in reducing motor fluctuations and total "off" time in patients. Oral drug administration has disadvantages such as risk of choking, poor absorption, and enzymatic degradation in the GI tract. Bioavailability is also altered by hepatic first-pass metabolism leading to unwanted metabolites that can contribute to side effects such as nausea, emesis, bowel obstruction, and dysphagia which limit the use of oral tablets. Some estimates suggest up to 50% of the general population have difficulty swallowing standard tablets and hard gelatin capsules which may lead to medication noncompliance issues [42]. These concerns have led to the development of novel drug delivery platforms through the skin, nasal , rectal, and oral mucosa. The Zydis® fast-dissolving drug delivery system is one such technology wherein a unique friable tablet is made by freeze-drying. It disintegrates rapidly in the mouth, releasing the drug into the saliva, and allowing for direct absorption of

the drug through the oral mucosa and sublingual route [43] while resolving problems with dysphagia and first-pass hepatic metabolism.

8.4.1 Summary of the Zydis Technology

The Zydis matrix is composed of many materials designed to achieve various objectives [42]. Polymers such as gelatin, dextran, or alignates are required to create a glassy amorphous structure that imparts strength and toughness during handling. Saccharides such as mannitol or sorbitol impart crystallinity, hardness, and elegance. Water is used in the manufacturing process to aid in the production of porous tablets which disintegrate rapidly on the tongue in 2–3 s. Preservatives, such as para-benzoic acids, at bacteriostatic concentrations are used to prevent microbial growth in the aqueous solutions during the manufacturing process. Suspending or flocculating agents, or both, for example gums, are used to minimize the sedimentation of dispersed drug particles during the manufacturing process and pH-adjusting excipients such as citric acid and sodium hydroxide are used to optimize the chemical stability of drugs, to alter the solubility of water-insoluble compounds, or to optimize the ionized fraction of a drug which is to be absorbed into the blood stream through pregastric membranes. Permeation enhancers such as sodium lauryl sulfate are used to optimize the transmucosal delivery of drugs absorbed through pregastric tissues, and collapse protectants such as glycine prevent the shrinkage of Zydis tablets during the freeze-drying process or during long-term storage. In addition, flavors and sweeteners are used to optimize taste, while microencapsulation polymers, such as cellulose derivatives, are used to mask the bitter taste of drugs, and coloring agents are used to give the product elegance and identity.

When the Zydis units are placed in the mouth, the freeze-dried structure disintegrates rapidly and releases the drug which dissolves or disperses into the saliva. The saliva containing the dissolved or dispersed medicine is then swallowed in the normal way. Some drugs can be absorbed from the mouth, pharynx, and esophagus as the saliva goes down to the stomach. In such drugs, the bioavailability from Zydis formulations can be significantly greater than standard swallow tablets. Other drugs that are not significantly absorbed from the pregastric region dissolve in the GI fluids and are absorbed in the conventional fashion. In such cases, the bioavailability of Zydis dosage forms is equivalent to that of the standard dosage form.

8.4.1.1 Drug Requirements

The Zydis formulations consist of drugs that are physically entrapped or dissolved in the fast-dissolving tablet matrix. Drugs suitable for the Zydis dosage form may have several characteristics. The dose of water-insoluble drugs may have an upper limit in order to retain the porous nature and fast dissolution feature of the matrix. The limitation on loading also helps avoid the drug being sensed in the mouth

as the tablet dissolves in the saliva. The dose of water-soluble drugs is desired to be <60 mg. However, the dose is governed by the behavior of the drug during freeze-drying and impact on tablet characteristics. For example, if eutectic mixtures are formed, these might either not adequately freeze or might melt at higher temperatures used during the drying cycle. The dissolved drug might also form an amorphous glassy solid on freezing which may collapse after drying because of sublimation of ice and loss of the supporting structure. These problems may be addressed by inclusion of a crystal-forming excipient to impart strength to the tablet matrix or using a nonaqueous solution of the active ingredient. Using these methods, Zydis units with large amounts of water-soluble drugs can be formulated. To prevent sedimentation of material during the manufacturing process, the particle size of insoluble drugs is desired to be less than 50 μm. The small particle size also helps reduce sensing the gritty texture of the drug. By using appropriate formulation and process techniques, however, high-quality Zydis tablets can be made using drug particles as large as 200 μm. In addition, the drugs must be chemically stable roughly over a 24-h period at room temperature; this may be necessary for preparing and storing the drug solution or suspension before starting the freeze-drying process. Drug products developed with the Zydis technology include oxazepam, lorazepam, loperamide, famotidine, loratadine, enalapril, phenylpropanolamine/brompheniramine, ondansetron, rizatriptan benzoate, risperidone, tepoxalin, clonazepam, and olanzapine.

8.4.1.2 Freeze-Drying Process

The solution or suspension is accurately dosed into pockets of large preformed blister packs to within 2% of the target weight. Once dosed, the water in the suspension is frozen by passing the blister trays through a liquid nitrogen-freezing tunnel. The frozen units are then loaded onto shelves of a large freeze-dryer to enable rapid removal of large volumes of vapor without melt-back. The drying process removes all ice by sublimation and the resulting Zydis tablets are porous.

8.4.2 Clinical Studies With Selegiline

Since the metabolites generated by first-pass metabolism of oral selegiline are responsible for several side effects, the pharmacokinetics of Zydis selegiline, and how it differs from the oral formulation are of significant interest. Clarke et al. [44] performed a series of studies to compare the pharmacokinetics of Zydis selegiline with the standard oral formulation. The mean C_{max} and mean $AUC_{0–inf}$ were not statistically different with Zydis 1.25 mg and oral selegiline 10 mg tablets; however, the concentration of the principal metabolites were at least 90% lower with the Zydis formulation as compared with the oral tablet. Similar results were reported by Seager (Fig. 8.3). In comparing three Zydis selegiline doses (1.25, 2.5, and 5 mg),

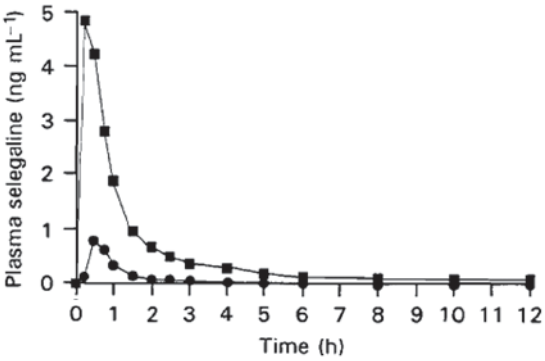

Fig. 8.3 Bioavailability of 10-mg selegiline from Movergan tablets (●) and from the Zydis dosage form (■) $n = m$ (From Ref [42] with permission from John Wiley and Sons)

there was a proportional increase in AUC_{0-inf} of selegiline and principal metabolites with dose. In one experiment, healthy volunteers were randomized to receive a single dose and 28 days of Zydis selegiline 1.25 mg, Zydis selegiline 10 mg, or oral selegiline 10 mg. Similar metabolite plasma concentrations were observed for Zydis selegiline 10 mg and oral selegiline 10 mg, but a significantly lower plasma concentration was observed for Zydis selegiline 1.25 mg ($p < 0.05$). Lower doses of selegiline with Zydis formulation can be used to furnish blood-level concentrations and therapeutic activities equivalent to higher-dose standard oral tablets (Fig. 8.4). The higher bioavailability and absorption is possible for water-soluble drugs such as selegiline because they have p*K*a values which enable the molecule to be in nonionized form at buccal pH in reasonable quantities in the saliva and is absorbed into the bloodstream though the membranes of the mouth, pharynx, and esophagus during the swallowing process [42].

Studies have also shown a large variability in the rate of absorption and metabolism between individuals with administration of oral selegiline [44–46]. Clarke et al. [44] evaluated the degree of pregastric absorption for Zydis selegiline in an open-labeled, randomized, three-way, single-dose, crossover study with 12 healthy

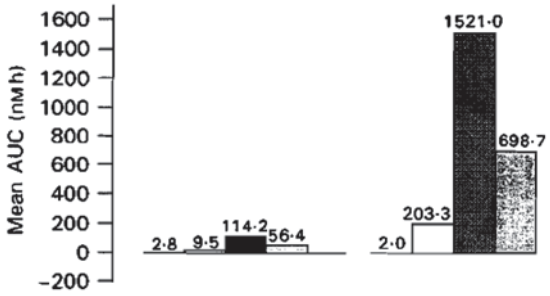

Fig. 8.4 Bioavailability of selegiline and selegiline metabolites (which have undesirable pharmacological activity) from 1.25-mg Zydis selegiline (*left*) and from 10-mg Movergan tablets (*right*): ■ selegiline; □ N-desmethylselegiline; ■ methamphetamine; ▒ amphetamine. (From Ref [42] with permission from John Wiley and Sons)

volunteers. Within 1 min of administration of Zydis selegiline 1.25 mg, 30% of selegiline was absorbed and no significant variability was observed between the volunteers. Faster absorption was also seen with the Zydis selegiline 1.25 mg compared to oral selegiline 10 mg tablets (T_{max} 0.25 vs. 0.5 h, respectively). The hepatic clearance of selegiline is dominated by the highly polymorphic CYP2B6 and CYP2C19 and could explain the variability in interindividual metabolism [47]. With almost 90% of an orally administered dose being metabolized before reaching systemic circulation, this variability may be responsible for poor medication response in some patients. Because the Zydis formulation significantly bypasses the first-pass metabolism, the drug plasma concentration is more stable and less impacted by interindividual enzyme variability.

The efficacy and patient preference of Zydis selegiline has been evaluated in a number of phase II and III clinical trials. An open-label, randomized study compared the therapeutic effects of conventional selegiline 10-mg tablets with Zydis selegiline 1.25 mg and Zydis selegiline 10-mg tablets in patients with PD who previously were receiving treatment with conventional selegiline tablets 10 mg daily as an adjunct treatment to levodopa or a dopamine agonist [48]. It was found that Zydis selegiline 1.25 and 10 mg were therapeutically equivalent to the conventional selegiline oral tablets. The patients also scored the formulation for salivation and swallowing problems along with taste and acceptability of the Zydis selegiline. The Zydis formulation was found to be well tolerated and preferred in 78% patients with a likeable taste. Another randomized, double-blinded, two-treatment-arm, placebo-controlled trial studied the use of Zydis selegiline as an adjunct therapy to levodopa to reduce "off" time in patients with PD [49]. A statistically significant reduction in percentage daily "off" time was observed at weeks 4–6 ($p=0.003$) and weeks 10–12 ($p<0.001$) in patients treated with Zydis selegiline compared with a placebo. The total number of "off" hours was reduced by 2.2 h/day as compared to 0.6 h/day in the placebo group.

An open-label, extension study was conducted to evaluate the long-term safety, efficacy, and tolerability of Zydis selegiline [50]. A total of 248 patients were recruited from previously reported randomized, double-blind, placebo-controlled, phase III trial by Waters et al. and a second identically designed trial [49, 51]. Patients were recruited after completion of 12-week entry trials and either started on Zydis selegiline 2.5 mg, if previously in the placebo group, or continued on Zydis selegiline 2.5 mg. The patients were evaluated at 4, 12, 24, and 40 weeks after the start of the extension study and every 6 months thereafter. A total of 89 patients completed at least 40 weeks of treatment with the maximum duration being 4.75 years in 4 patients. The average reduction in daily "off" time was 8.1% (1.4 h).

In the randomized, placebo-controlled trial, there was no significant difference in adverse events between the groups administered Zydis selegiline and placebo [49]. The most common adverse events reported were similar to known complications of levodopa therapy such as dizziness, dyskinesia, hallucinations, headache, and dyspepsia.

Zydis selegiline also does not cause a tyramine pressor effect. In an open-label safety study [52], 24 healthy volunteers were randomized to receive Zydis selegiline

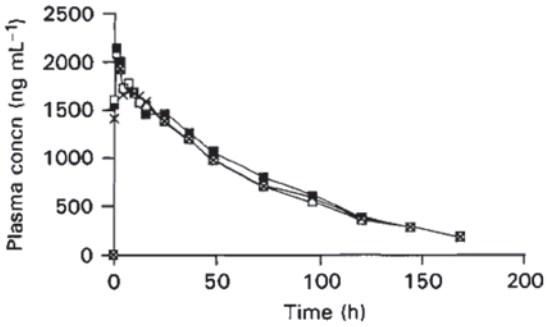

Fig. 8.5 Bioavailability of 20-mg piroxicam from feldene capsules (■) and from the Zydis dosage form with (□) and without (□) water. (From Ref [42] with permission from John Wiley and Sons)

1.25 mg or selegiline tablets 10 mg for 14–16 days. A pressor effect was apparent with 400-mg tyramine both before and after 14 days of treatment with Zydis selegiline, whereas the threshold dose required to elicit the same response with oral selegiline tablets was 200 mg ($p < 0.0001$).

8.4.3 Absorption of Piroxicam

In drug molecules that are water insoluble, such as piroxicam, the overall dissolution rate from the Zydis dosage forms in the GI fluids is similar to the standard dosage form, and the bioavailability of Zydis units is therefore equivalent to the standard tablet or hard gelatin capsule dosage form. A typical plasma concentration curve for a Zydis formulation compared with a standard oral dosage form for such molecules is shown in Fig. 8.5.

8.4.4 Advantages of ODT Technology

The Zydis technology provides an alternative form of drug delivery that is easier to administer (dissolves fast in the mouth), has increased absorption, and enhanced bioavailability. The new formulation eliminates the risk of choking and difficulty with swallowing, improving patient convenience, and ultimately compliance. Dysphagia and choking symptoms are often worse in the "off" state, which is also when they need to administer oral medications and can lead to increased risk of mortality in patients. Since the Zydis formulation uses pregastric absorption it does not need water or swallowing for drug delivery, and with at least 30% of drug being absorbed under 1 min, it is a practical and convenient medication for many PD patients [44]. Active metabolites that are generated by first-pass hepatic metabolism can reduce the usefulness of traditional oral selegiline. The active metabolites of selegiline have been known to contribute to or worsen sleep problems, which are common in PD. The Zydis selegiline reduces metabolites by 90% and can therefore be safely used with PD patients with insomnia [44]. Zydis selegiline has also been shown

to allow for higher serum levels of selegiline and increased monoamine oxidase B (MAO-B) activity without causing hypertension or tachycardia, more favorably than oral selegiline [41].

8.5 Fentanyl

8.5.1 Actiq® Lozenge

Breakthrough pain is understood as a transitory flare of pain that occurs in many cancer patients against a background of otherwise controlled, persistent pain [53]. The treatment of breakthrough pain is a challenging task. Immediate release short-acting oral opioids that are taken as needed are frequently used to treat breakthrough pain. In cancer patients, these include morphine sulfate, oxycodone, hydrocodone, and hydromorphone [54]. Oral immediate release morphine has long been considered the gold standard in treatment for cancer breakthrough pain. However, its relatively long time to analgesic onset, delay in maximal analgesic effect, and prolonged duration of action make it unsuitable for the management of breakthrough pain episodes. Breakthrough pain needs to be treated with an opioid in a formulation with an analgesic profile that closely matches the characteristics of a breakthrough pain episode [55]. Oral transmucosal fentanyl citrate (OTFC, brand name: Actiq®) is the first medication that was developed specifically for the treatment of breakthrough pain. It is designed to provide fentanyl, the opioid, via a unique OTM delivery system and offers patients personal pain control. In contrast to the 30-min onset of action of oral morphine, Actiq starts to relieve pain within 5 min of administration [55]. Similarly, Actiq's duration of action of 1–2 h is shorter than the typically 4 h seen with oral morphine, which reduces the risk for needlessly prolonging the treatment for episodes of breakthrough pain.

OTFC is a solid formulation of fentanyl citrate, a potent synthetic opioid (50–100 times as potent as morphine) that is lipophilic, short acting, and has rapid onset, with a selective activity for μ-receptors expressed in the brain, spinal cord, and other tissues. The OTFC is formulated as a sweetened solid drug matrix on a handle (lozenge) that the patient can rotate in the mouth for optimal absorption and remove the unit if excessive opioid is being administered. This makes it an effective treatment for cancer patients who already receive opioids and experience flares of pain. OTFC is available in six strengths equivalent to 200, 400, 600, 800, 1200, and 1600 μg fentanyl base.

8.5.1.1 Pharmacokinetics

The fentanyl in the OTM dosage form is delivered through a combination of initial rapid absorption from the buccal mucosa and a more prolonged absorption of swallowed fentanyl from the GI tract [56]. Under normal conditions, about 25%

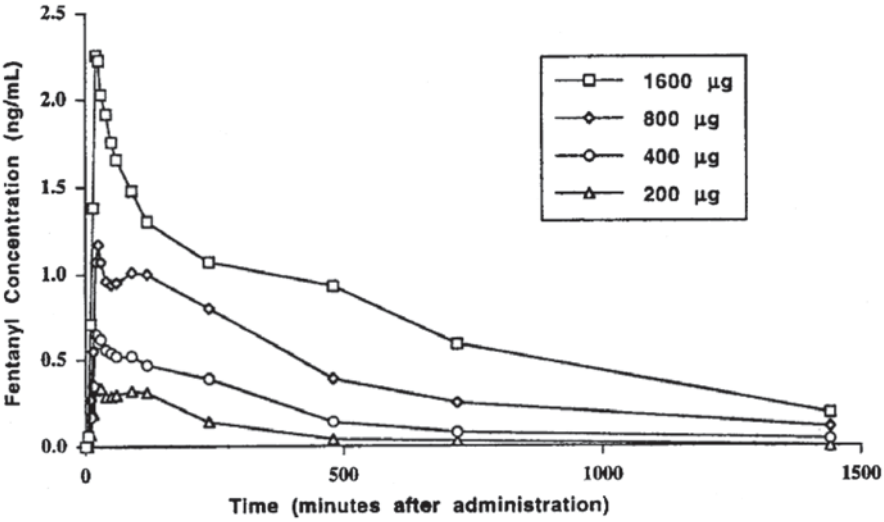

Fig. 8.6 Mean serum fentanyl levels following administration of the four strengths of OTFC (200, 400, 800, and 1600 mcg units) in adult subjects. OTFC oral transmucosal fentanyl citrate. (From Ref [57] with permission from Wolters Kluwer Health)

Table 8.1 Pharmacokinetic parameters of various strengths of oral transmucosal fentanyl citrate (OTFC; 200–1600 mcg) from dose-proportionality study. (From Ref [57] with permission from Wolters Kluwer Health)

Dose, mcg	T_{max} (min) Median	C_{max} (ng/mL) Mean ± SD	AUC_{0-1440} (ng/mL*min) Mean ± SD	$t_{1/2}$ (min) Mean ± SD
200	40	0.4 ± 0.1	172 ± 96	193 ± 93
400	25	0.8 ± 0.2	400 ± 363	386 ± 443
800	25	1.6 ± 0.5	887 ± 859	381 ± 211
1600	20	2.5 ± 0.6	1508 ± 1360	358 ± 162

SD standard deviation

of the total dose is rapidly absorbed through the buccal mucosa, avoiding first-pass metabolism, and becomes systemically available. The remaining 75 % of the total is swallowed with the saliva and then slowly absorbed from the GI tract. The blood fentanyl profile and the bioavailability of fentanyl varies, depending upon the fraction of the dose absorbed through the oral mucosa and the fraction swallowed. About one third of the dose swallowed (25 % of the total dose) escapes hepatic and intestinal first-pass elimination and becomes systemically available. Thus, the generally observed 50 % bioavailability of OTFC is roughly equally divided between rapid transmucosal absorption and the slower GI absorption. Dose proportionality across the range of strengths (200–1600 mcg) has been demonstrated in a balanced crossover design study [57]. Figure 8.6 shows the mean serum fentanyl levels at four doses of the OTFC. The pharmacokinetics parameters obtained from the dose proportionality study are shown in Table 8.1.

The mean C_{max} ranged from 0.4 to 2.5 ng/mL. The median time to maximum plasma concentration (T_{max}) varied from 20 to 40 min as measured from the start of administration. In addition, studies showed that two smaller doses of OTFC (400 mcg) administered simultaneously are pharmacokinetically equivalent to the administration of a single dose of 800 mcg [58].

8.5.1.2 Clinical Studies

OTFC for management of breakthrough pain has been evaluated in small, short-term studies in adult patients with cancer-related pain. In these studies, patients were taking an oral opioid (usually morphine) or transdermal fentanyl as their ongoing medication to control persistent pain. Two randomized, double-blind dose titration studies of OTFC have been reported ($n=65$ and 62) [59, 60]. The results show that 74 and 76% of patients, respectively, were able to identify a safe and effective dose of OTFC. The mean preferred dose of OTFC was approximately 600 mcg. However, no relationship was found between the preferred dose of OTFC and the daily dose of ongoing opioid in either study. This indicates that the optimal dose of OTFC is not related to the daily dose of the fixed schedule opioid. These studies also included open-label comparisons of OTFC and the patients on regular oral opioids used for breakthrough pain. Although neither study was specifically designed to compare the analgesic efficacy of OTFC to the regular rescue drug, OTFC was reported to produce a greater analgesic effect, better global satisfaction, and a more rapid onset of action than the usual breakthrough pain medication [59, 60].

The effectiveness of OTFC has been evaluated in one randomized, placebo-controlled trial [61] and one randomized, comparative study with immediate release morphine sulfate (IRMS) [62]. The placebo-controlled trial was a multicenter, crossover study that evaluated the efficacy of individualized doses of OTFC. A total of 130 patients went through an open-label dose titration to identify their successful dose, of which 92 patients consented to participate in the randomized double-blind study. The primary efficacy analysis indicated that the analgesic effect in terms of pain intensity difference and pain relief were significantly greater with OTFC than with placebo at all time points measured after consumption of OTFC ($p<0.0001$). The mean global performance evaluation values also showed a significant preference for OTFC ($p<0.0001$) [61]. The comparative study was a randomized, double-blind, crossover study to assess the efficacy of successful doses of OTFC with IRMS. At first, 134 patients who were using a dose of 15–60 mg IRMS were entered into an open-label dose titration study to identify a successful dose of OTFC, of which 93 patients entered the randomized double-blind phase study. The primary efficacy analysis indicated that OTFC was significantly superior to IRMS in terms of pain intensity difference ($p<0.008$) and pain relief ($p<0.009$) at each time point and global performance rating ($p<0.001$). Moreover, significantly ($p<0.001$) more pain episodes treated with OTFC had a greater than 33% reduction in pain intensity at 15 min than IRMS, which indicates a faster onset of action for the OTFC [62].

Another open-label study studied the long-term safety and tolerability of OTFC in ambulatory cancer patients with breakthrough pain [63]. Overall, 41,766 units of OTFC were used to treat 38,595 episodes of breakthrough pain in 155 patients. Patients had previously been titrated to a successful OTFC dose before the study. About 92% of episodes were successfully treated with OTFC with no trending observed toward decreasing effectiveness over time. The majority of the patients (61%) did not require dose escalation during treatment. Global satisfaction ratings were consistently above 3 (0 = poor, 4 = excellent) implying very good to excellent pain relief. Adverse events commonly found with OTFC include somnolence (9%), constipation (8%), dizziness (8%), nausea (8%), and vomiting (5%). There were no reports of abuse and patients and their families did not raise any concerns about the drug's safety. OTFC was found to be safe and effective for long-term treatment of breakthrough pain in cancer patients at home.

In one study, the efficacy of OTFC was evaluated for outpatient management of severe cancer patient crises [64]. Before the OTFC treatment, all patients reported a mean pain intensity of 9.0 (standard deviation (SD) = 1.2). After OTFC treatment, patients reported a mean intensity of 3.0 (SD = 1.4), indicating a significant reduction in pain intensity ($p < 0.001$). In the majority of the cases, OTFC averted the need for an emergency center visit, parenteral opioids, and admission to the hospital, which demonstrates that OTFC can be an effective alternative over IV opioids to rapidly relieve pain in opioid-tolerant cancer patients with breakthrough pain.

8.5.1.3 Non-cancer Pain

In addition to breakthrough pain, OTFC has been used in a variety of clinical situations involving noncancer pain.

In one randomized study, 133 postoperative patients were given one dose of either OTFC (200 or 800 mcg) and a placebo IV injection or IV morphine (2 or 10 mg) and an OTM placebo unit [65, 66]. The OTFC was compared with IV morphine in terms of pain relief, pain intensity, time to pain relief, and time to remedication and it was found that OTFC produced rapid pain relief comparable to IV morphine. In addition, the larger dose of OTFC (800 mcg) produced better and more sustained pain relief.

Two double-blinded comparative studies evaluated the analgesia for pediatric burn wound care on inpatient and outpatient basis with OTFC compared to oral hydromorphone, and oral oxycodone, respectively [67, 68]. The OTFC showed much improved pain scores before wound care and better anxiolysis during wound care compared to hydromorphone, with similar results and improved palatability compared to oxycodone.

In one placebo-controlled pediatric study, OTFC or placebo was administered to children referred for bone marrow aspiration and lumbar puncture [69]. The OTFC resulted in significant reduction in pain ratings and median pain scores were reduced to tolerable levels.

In a study with 18 patients having acute, refractory migraine headaches who had been treated as outpatients with opioid therapies, self-administration of OTFC at home rapidly and significantly relieved migraine pain (an average of 75 % reduction in pain intensity at 120 min post administration) [70]. It also prevented the need for a visit to the emergency department and resulted in high patient satisfaction ratings (94 % patients reported satisfaction with their treatment).

In one study with patients experiencing moderate-to-severe pain from sickle cell disease, each subject was prescribed a long-acting opioid in combination with OTFC for breakthrough pain [71]. With this pharmacotherapy protocol, pain was well controlled and emergency hospital visits and admissions were drastically reduced (reduction to less than or equal to 1 visit, down from 6 to 18 visits per year).

8.5.2 Fentora®/Effentora®

As discussed in the preceding section, Actiq is a safe and effective treatment for management of breakthrough cancer pain and is preferred by patients over other opioid dosage forms. Nonetheless, this product has some limitations [54]. Differences in application technique at the oral mucosa may result in variable absorption of the fentanyl dose. The absorption may also reduce if the patient has reduced saliva volume, applies the OTFC to the tongue and gums instead of buccal mucosa, chews the product, has ingested liquids that change the oral pH before product application, or applies the product for less than, or longer than, 15 min. In order to overcome these limitations, other transmucosal fentanyl formulations have been developed. Recently, five products, Effentora®/Fentora®, Abstral®, Instanyl®, Breakyl®/Onsolis™, and PecFent® have been concurrently approved in Europe and/or the USA and have documented efficacy in quickly relieving breakthrough pain. In this section, the oral formulations (Effentora/Fentora, Abstral, Breakyl/Onsolis) will be discussed in greater detail. The other two products Instanyl and PecFent are intranasal fentanyl sprays and are out of the scope of this chapter.

Effentora/Fentora is an effervescent buccal tablet formulation of fentanyl citrate that is to be placed in the buccal cavity above a rear molar between the cheek and gum, and retained in position until it disintegrates after 14–25 min [72, 73]. The fentanyl buccal tablet is formulated on the OraVescent® drug delivery technology that produces transient pH changes and optimizes dissolution, permeation, and absorption of fentanyl through the buccal mucosa. Recently, the Fentora label was changed to incorporate sublingual dosing since a bioequivalence study between the buccal and sublingual dosing was successful.

Abstral is a sublingual mucoadhesive fentanyl tablet that rapidly disintegrates while in contact with sublingual mucosa into an ordered mixture of fentanyl in a soluble carrier [74]. The formulation enables the fentanyl to dissolve rapidly and take advantage of the known high permeability of the sublingual mucosa leading to the absorption of the fentanyl dose in approximately 30 min. The tablet is placed

Table 8.2 Pharmacokinetic parameters of newer transmucosal fentanyl solid dosage forms. The data shown are mean ± SD (where available) unless stated otherwise. (From Ref [54] with permission from Springer)

Parameter	Effentora®/ Fentora® buccal tablet	Abstral® sublingual mucoadhesive tablet	Brekyl®/Onsolis™ buccal soluble film	Actiq® oral transmucosal lozenge
Absolute bioavailability (%)	65 ± 20	70	71	50
T_{max} (min)	34.8 (20, 180)[a]	56.7 ± 24.6	90 (45–240)[b]	20[c]
C_{max} (ng/mL)	0.97 ± 0.53	0.91 ± 0.3	1.33 ± 0.31	1.6 ± 0.5
AUC_{inf} (ng*h/mL)	4.72 ± 1.95	290.8 ± 92.5	13.03 ± 3.45	887 ± 859
Dose linearity	Yes	Yes	Yes	Yes
$t_{1/2}$ (h)	11.09 (3.44, 20.59)[a]	5.4 ± 1.7	19.03 ± 8.31	6.35 ± 3.52

[a] Median (90% CI)
[b] Median (range)
[c] Median (range not reported)
AUC area under the curve

below the tongue at the deepest part and held in place until it completely dissolves in the sublingual cavity.

Breakyl®/Onsolis™ is a fentanyl buccal soluble film formulation based on the BioErodible MucoAdhesive (BEMA™) technology [75, 76]. Breakyl®/Onsolis™ film is applied onto the buccal mucosa and it releases the drug into the oral membrane. The film dissolves in 15–30 min after application. Due to the nature of the dosage form, the amount of fentanyl delivered transmucosally is proportional to the film surface area.

The pharmacokinetic parameters of these new transmucosal formulations of fentanyl are presented in Table 8.2.

8.5.2.1 OraVescent® Technology

In the OraVescent technology (CIMA Labs, Inc. Brooklyn Park, MN, USA), a dynamic shift in the pH is produced to facilitate the dissolution followed by absorption of a drug [77]. In the case of Fentora/Effentora, an initial reduction in pH in the microenvironment where the tablet is placed near the buccal mucosa favors the dissolution of ionized fentanyl. Once the fentanyl is dissolved, when the pH starts increasing, the nonionized fentanyl is favored, which, as it forms, is readily absorbed. The dynamic pH variations enhance the extent and speed of absorption of fentanyl across the buccal mucosa. The OraVescent buccal tablets utilize effervescence to bring about the dynamic change in pH necessary for rapid drug delivery. The ef-

fervescence reactions produce carbon dioxide from the combination of an acid and bicarbonate or carbonate in an aqueous solution. The protons combine with the bicarbonate/carbonate and carbonic acid is formed. Carbonic acid, being unstable, rapidly disassociates into carbon dioxide and water. In open systems, the carbon dioxide escapes into the atmosphere and therefore the drive to equilibrium favors the continuous consumption of the acid. Fentora/Effentora tablets contain citric acid, sodium bicarbonate, sodium carbonate, and fentanyl citrate. As the tablet beings to disintegrate, at first the citric acid dissolves and lowers the pH. The hydrogen ions formed combine with bicarbonate and carbonate ions and produce carbon dioxide. As the carbon dioxide escapes, the pH increases, leaving sodium citrate and excess sodium bicarbonate in the solution.

Durfee et al. [77] reported an interesting study that demonstrated the dynamic pH changes with in vitro data. A system was developed to measure pH changes on the surface as the tablet dissolved. A pH paper was placed over a tablet held between two microscope slides and a drop of deionized water was applied to the paper. The water rapidly spread through the paper and wetted the surface of the tablet. As the tablet dissolved, the pH paper was digitally photographed at specific times. The pH over distinct regions of the paper were then determined from digital images and comparison to reference pH standards. The average pH over the entire tablet surface was also determined. Figure 8.7 shows the local pH changes on the surface as the tablet dissolved at times 10 s, 1, 3, and 5 min after the drop of water was placed.

Initially, the citric acid dissolved and the pH dropped, as indicated by the red areas. The average pH dropped to minimum of 5.0 at 10 s. The low pH favors the dissolution of fentanyl citrate with the formation of the ionized form of fentanyl which is a basic drug with p*K*a ~ 7.3. At low pH, the concentration of the ionized portion of the drug may be as much as ten times higher than what it would be at neutral pH [78]. In this scenario, permeation of the ionized drug is promoted. As the carbon dioxide produced is dissipated, the pH rises (shown by blue areas in Fig. 8.7) and the nonionized form of fentanyl is favored. The dissolved fentanyl converts to the nonionized form and the solution may even get supersaturated. Since recrystallization is a slow process, the nonionized form (which is more lipophilic) is pushed into the buccal mucosa. Thus, the drug is delivered through both the ionized and nonionized form in the OraVescent technology. A similar pH profile has been reported when the tablet dissolves in artificial saliva (i.e., phosphate-buffered saline (9.8 mmol/L phosphate, 150 mmol/L chloride), given that the buffer capacity of saliva is much lower than the buffer capacity of the high electrolyte concentration at the surface of the tablet [77].

8.5.2.2 Clinical Studies

The efficacy of the OraVescent® technology as compared to a noneffervescence system has been demonstrated in clinical studies. In one study, 12 healthy volunteers were administered three different formulations of buccal tablets on a randomized schedule [79]:

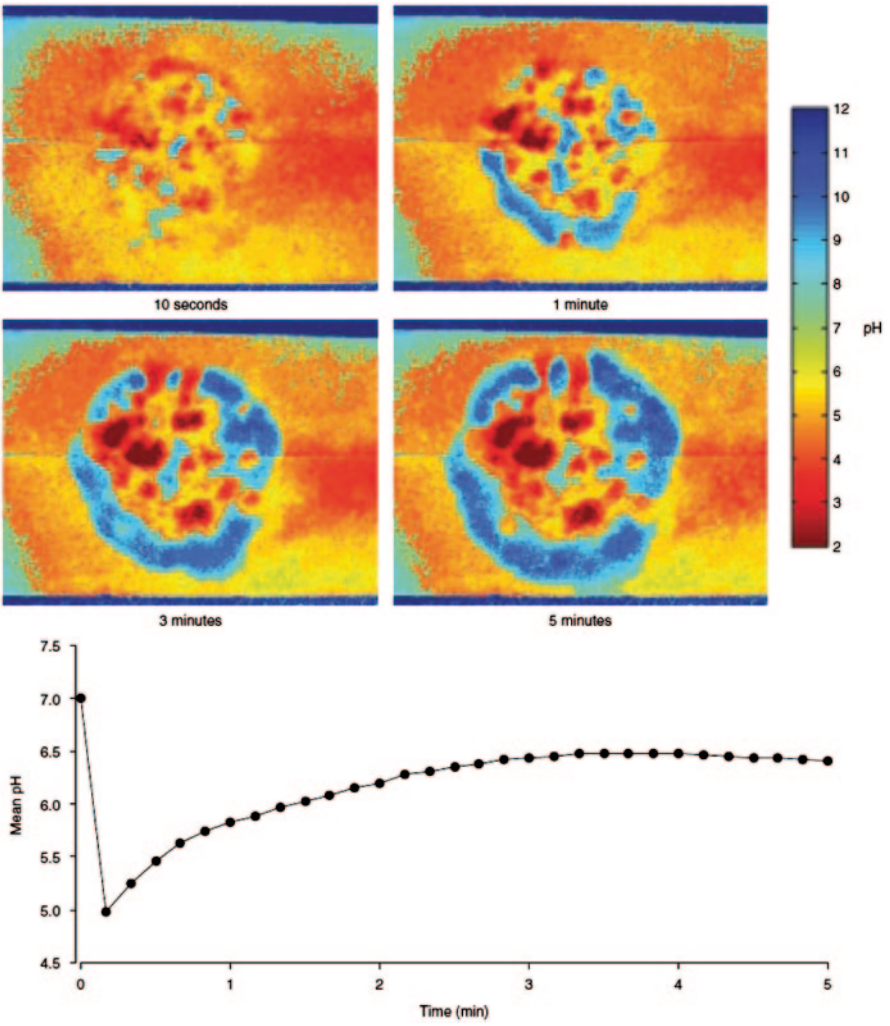

Fig. 8.7 The top of the figure contains computer-enhanced color images of pH paper placed over a fentanyl effervescent buccal tablet. The images were taken at 10 s, 1, 3, and 5 min after the pH paper was wetted with water. To the right of these images is a color reference for pH values from 2 to 12 obtained by fitting a calibrated computer model to the original digital images. Below these images is a graph of the average pH over the surface of the dissolving tablet as it changed over 5 min. (From Ref [77] with permission from Springer)

a. OraVescent buccal tablets containing 200 mcg fentanyl
b. A 200-mcg fentanyl tablet similar to the above OraVescent tablet but without citric acid, sodium bicarbonate, and sodium carbonate (the effervescence system)
c. The oral transmucosal 200 mcg fentanyl citrate lozenge (OTFC; Actiq®)

Subjects receiving treatment A and B placed the tablet between the upper gum and cheek, above a premolar tooth, and left it in place for 10 min. After this time

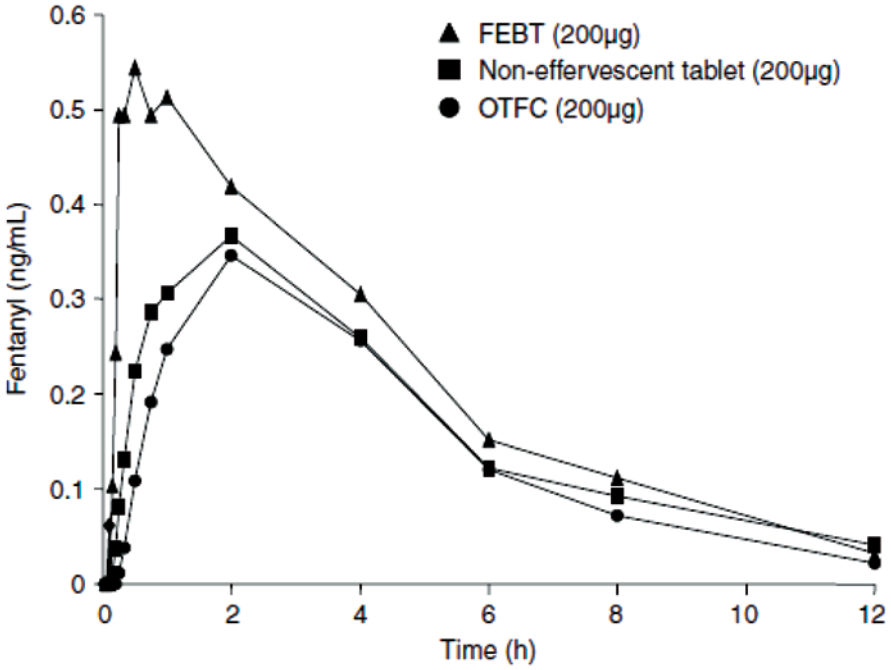

Fig. 8.8 Serum fentanyl concentrations after administration of fentanyl effervescent buccal tablets (*FEBT*) 200 mcg, fentanyl tablets without effervescent agents 200 mcg, and oral transmucosal fentanyl citrate (*OTFC*) 200 mcg. (From Ref [79] with permission from Drug Development and Delivery)

elapsed, if a subject felt that any portion of the tablet was undissolved, they were told to gently rub the outer cheek over that area for 5 min. If any tablet portion remained after 15 min, the subjects were instructed to let it dissolve on its own without further manipulation. The OTFC was administered by placing the lozenge between the lower gum and cheek. The unit was moved from side to side inside the mouth until all the lozenge was consumed in approximately 15 min. The subjects were instructed to suck and not chew on the unit.

The serum fentanyl concentrations with time are shown in Fig. 8.8 and the pharmacokinetic parameters are summarized in Table 8.3. It can be seen that fentanyl absorption from the OraVescent delivery system is superior than the nonefferves-cent or OTFC formulation. The OraVescent® tablets displayed a higher peak serum concentration of ~0.6 ng/mL compared to ~0.4 ng/mL for OTFC ($p < 0.001$). Fentanyl absorption with OraVescent technology was more rapid (shorter median T_{max} among all three formulations, $p < 0.003$) and the bioavailability was highest (AUC from time zero to T_{max} is about 1.47 times the corresponding value with OTFC) $p < 0.01$.

Table 8.3 Pharmacokinetic parameters from single-dose (200 mcg) study comparing OraVescent fentanyl buccal tablets (OFBT) with noneffervescent fentanyl tablets and oral transmucosal fentanyl citrate (OTFC). (From Ref [77] with permission from Springer)

Parameter	OFBT	Noneffervescent tablet	OTFC	*p* value
C_{max} (ng/mL) Mean and SD	0.64 ± 0.28	0.40 ± 0.07	0.41 ± 0.15	< 0.001
AUC_{0-Tmax} (ng*h/mL) Mean and SD	2.66 ± 0.63	2.04 ± 0.87	1.81 ± 0.94	< 0.01
Median T_{max} (h)	0.5	2	2	< 0.003

OFBT OraVescent fentanyl buccal tablets, *OTFC* oral transmucosal fentanyl citrate

Similar enhanced pharmacokinetic parameters for the OraVescent® fentanyl buccal tablets have been displayed in other studies involving healthy volunteers [80, 81]. For example, one study compared the following formulations,

a. 400 mcg fentanyl OraVescent® tablets administered transmucosally
b. 800 mcg fentanyl OTFC lozenge administered transmucosally
c. 800 mcg fentanyl OraVescent® tablet administered orally
d. 400 mcg fentanyl administered intravenously (comparator)

Comparing the three test products, the results showed that formulation A had the highest bioavailability (0.65) followed by formulation B (0.47), followed by formulation C (0.31). The T_{max} was also smallest for formulation A (47 min), followed by formulation C (90 min), followed by formulation B (90 min). The calculations show that approximately 30 % smaller doses will be required in the OraVescent buccal tablets to achieve equivalent bioavailability to the OTFC lozenge formulation. The reduced T_{max} values for OraVescent buccal tablets have also been confirmed in other multiple dose studies, as well as in dose proportionality studies regardless of the administered dose.

The dose proportionality with OraVescent buccal tablets has been studied in healthy volunteers across the potential therapeutic range (200–1080 mcg fentanyl) [82]. The AUC from time zero to infinity was linear with dose across the range, whereas C_{max} increased linearly from 200 to 800 mcg. At the same time, the increase in C_{max} was smaller than 20 % as compared to proportional at 1080 mcg dose. Other studies focusing on 100–800 mcg fentanyl dose have also confirmed the dose proportionality in OraVescent buccal tablets [83–85].

8.5.2.3 Treatment of Breakthrough Pain

To demonstrate that the OraVescent technology provides superior onset of analgesia, fentanyl OraVescent buccal tablets were administered to opioid-titrated patients with cancer who also suffer from breakthrough pain [86, 87]. Pain intensity and

relief were recorded at 15, 30, 45, and 60 min post administration and patient ratings of global medication performance were noted at 30 and 60 min. The analgesic effect of fentanyl from OraVescent buccal tablets was reported as early as 15 min and the relief lasted for 60 min. Similar results confirming the enhanced pharmacokinetics of the fentanyl OraVescent buccal tablets were reported in another well-designed phase III study with cancer patients suffering from breakthrough pain [88]. A single dose of fentanyl OraVescent buccal tablet (100–800 mcg) provided clinically significant pain relief within 15–60 min after administration [88].

8.6 Nicotine

8.6.1 Commit® Lozenges

The role of nicotine in tobacco dependence has been well studied and this led to the development of the nictone replacement therapy (NRT) for smoking cessation [89]. Nicotine undergoes first-pass metabolism in the liver, which reduces the overall bioavailability of swallowed nicotine pills. A pill that may produce high-enough nicotine levels in the plasma would risk causing adverse GI effects. To overcome this problem, NRT products are available in several forms such as transdermal patches, inhalator, nasal spray, sublingual tablets, gums, and lozenges. Nicotine gum is available at 2 and 4 mg strengths, and nicotine lozenges are available in 1, 1.5, 2, and 4 mg strengths.

Popular dosage forms for NRT include the Commit® lozenge by GlaxoSmith-Kline, who also manufacture the Nicoderm CQ® patches and Nicorette® gum, and the Nicotinell® lozenges made from nicotine bitartrate (Novartis Consumer Health). The active ingredient in Commit lozenge is nicotine polacrilex and the inactive ingredients include aspartame, flavor, magnesium stearate, calcium polycarbophil, potassium bicarbonate, sodium alginate, mannitol, sodium carbonate, and xanthum gum. Nicotine polacrilex is a complex formed between nicotine and a weak carboxylic cation exchange resin. The nicotine polacrilex lozenge is a hard tablet that is placed in the buccal cavity and exploits the known safety and efficacy of buccal transmucosal delivery of nicotine. The lozenge slowly erodes in the buccal cavity due to physical abrasion and nicotine is released over approximately 30 min [90]. When the lozenge comes in contact with moisture (i.e., saliva), salt ions displace nicotine from the ion-exchange resin and activate its release in the oral cavity. In the alkaline pH of saliva and the solubilization of potassium bicarbonate and sodium carbonate, nicotine exists mainly in its free base form and is absorbed by the buccal route or is ingested. This slow release and absorption compares starkly with the release from nicotine gums which is controlled by the force and frequency of chewing [91].

8.6.1.1 Clinical Studies

A number of clinical studies report the efficacy of the nicotine polacrilex lozenge compared to other dosage forms and conclude that using NRT increases the success of smoking cessation by 50–70 % as compared to not using NRT or using a placebo [89, 90, 92]. One study compared the pharmacokinetic parameters of the nicotine polacrilex Commit lozenge with the Nicorette gum in healthy adult smokers with four separate clinical trials. The first two studies were single-dose studies that compared the pharmacokinetic characteristics of the 2- and 4-mg lozenge with the gum. The third study was a multiple dose study that investigated the effect of different dosing intervals to the lozenge and the gum at both the 2- and 4-mg doses. A fourth study investigated the effect of improper use of the lozenge, such as nicotine absorption after chewing the lozenge as compared to using the lozenge as directed.

In the single-dose comparison study at the 4-mg dose level, both the C_{max} and T_{max} values of the lozenge were greater than the gum (C_{max} of 10.8 vs. 10.0 ng/mL; T_{max} of 1.1 vs. 0.9 h, respectively) [90]. In both dosage forms, nicotine plasma concentrations reduced with comparable elimination half-lives (average of 2.3 vs. 2.1 h for the lozenge and gum, respectively). The mean AUC_{0-inf} for the lozenge was 27 % larger than for the gum (44.0 vs. 34.6 ng.h/mL, respectively) possibly because the gum does not release all of the nicotine whereas the lozenge does not have this limitation. The AUC_{0-inf} ratio from the mean values for the lozenge and gum was 1.3 (SD = 0.6). The time to complete lozenge dissolution was 33 min (SD = 4, range = 27–41).

In the single-dose comparison study at the 2-mg dose level, the T_{max} occurred significantly later for the lozenge than the gum (average of 1.0 and 0.75 h, respectively; $p = 0.020$) [90]. The 90 % confidence interval of the lozenge to gum AUC_{0-inf} ratio was 1.15–1.45 with a mean of 1.35 (SD = 0.46). The 90 % confidence interval of the lozenge to gum C_{max} ratio was 0.97–1.22 with a mean of 1.14 (SD = 0.39). The time to complete lozenge dissolution was 20.8 min (SD = 1.3, range = 19–24).

In the multiple dose study with different dosing intervals, it was found that dosing lozenges every 90 min showed a lower plasma concentration compared with gums dosed every 60 min for both the 2- and 4-mg doses. Finally, the study that investigated the effect of using the lozenge as instructed in the label versus chewing and swallowing, and chewing and retaining in the mouth for 5 min and then swallowing found some interesting results. The C_{max} and AUC achieved by using the lozenge as instructed were higher than both the methods that involved chewing onto the unit, suggesting that using the lozenge differently than the label would not result in faster or higher nicotine levels and this concern is unfounded [90]. The nicotine lozenge has also been shown to be effective for relieving craving and partially effective for relief of withdrawal. In both low- and high-dependency smokers, treatment with the lozenge showed lower craving within the first 2 weeks of craving when it is at the peak [93, 94]. These results starkly contrast with that for the sublingual tablet which only reduced craving and withdrawal in high or moderately dependent smokers [95, 96]. In the highly dependent smokers, the 4-mg lozenge was particularly robust in suppressing withdrawal symptoms and craving even in the second

week of abstinence, producing high quit rates, reducing weight gain, and showed a significant difference between placebo groups and active-treated groups [93, 94]. In conclusion, the nicotine lozenge has been demonstrated to be an effective and safe route for smoking cessation.

8.6.2 OraVescent® Nicotine Product

CIMA Labs, Inc (Brooklyn Park, MN, USA) has also explored the advantages of the OraVescent® technology with other drug molecules. One such product is an OraVescent Nicotine buccal tablet which delivers nicotine more effectively than other dosage forms. The mechanism of action is the same as the OraVescent fentanyl in that an effervescent couple and pH modifier are used to enhance the transmucosal delivery of nicotine. The dosage form can be placed in a number of locations, including but not limited to, buccally, gingivally, and sublingually. Since the OraVescent tablets are much smaller than the Nicotine lozenges, they could increase user friendliness and patient compliance, for example, once a tablet is placed in the buccal cavity, the patient can carry on with normal activities, such as talking. The tablet can be taken discreetly without anyone knowing that they have a tablet in their mouth. For example, in one embodiment, the 2-mg nicotine OraVescent tablet weighed 200 mg while the Commit® Nicotine lozenge for the same dose weighs 1225 mg.

8.6.2.1 Clinical Studies

One open-label, single-dose (2 mg), randomized five-way crossover clinical study in healthy adult smokers compared the effectiveness of (1) nicotine polacrilex in OraVescent technology, (2) nicotine bitartrate in OraVescent technology, (3) Commit lozenge, (4) nicotine polacrilex in OTM tablets (without any ingredients essential for the OraVescent technology), and (5) nicotine bitartrate in OTM tablets (without any ingredients essential for the OraVescent technology). There were 20 patients in each arm of the study. The plasma nicotine concentration with hours from dosing is shown in Fig. 8.9.

The results demonstrate the superior delivery of nicotine using the OraVescent technology. The OraVescent nicotine formulation shows a significantly shorter T_{max}, higher C_{max}, and higher AUC (0–T_{max}) than the commercially available Commit lozenge as well as noneffervescent formulations. Thus, with the OraVescent technology, the nicotine can be delivered more rapidly, which can help reduce the "craving" period that can often determine the success or failure of a nicotine cessation product or program. The OraVescent nicotine can also deliver the same therapeutic levels of nicotine using a smaller dose that can help relieve patient GI discomfort as a smaller amount of nicotine is likely to be swallowed.

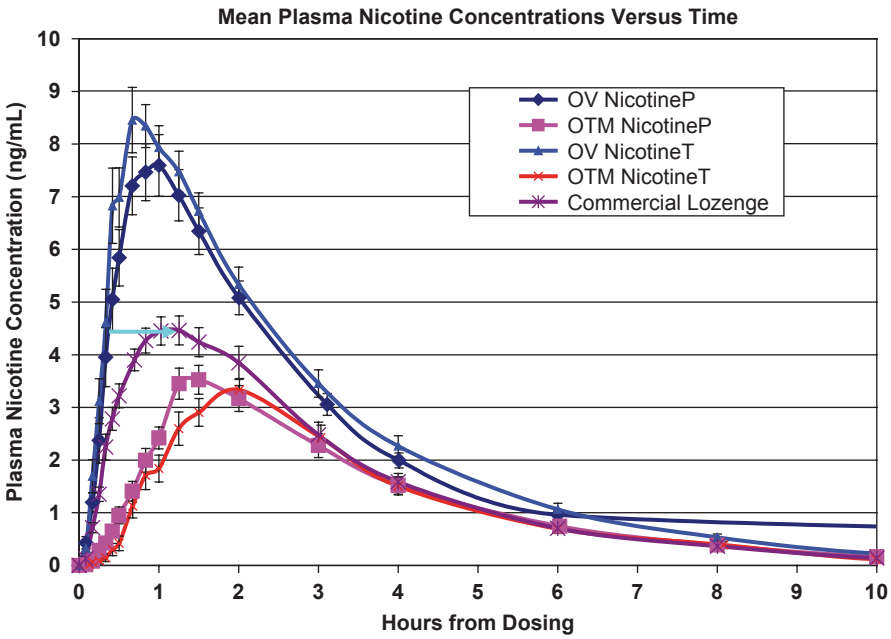

Fig. 8.9 Mean plasma nicotine concentration versus time for five formulations; *OV* OraVescent, *OTM* Oral transmucosal w/o effervescence

8.7 Summary

Oral mucosal drug delivery offers many advantages over conventional dosage forms especially in situations where rapid onset of drug action is necessary and extensive first-pass GI and/or hepatic metabolism exists. In certain situations, metabolites may present undesirable side effects, and transmucosal delivery of drugs can be a safer choice whereas also resulting in higher bioavailability. The field has progressed significantly with deeper understanding of the basic mechanisms of drug delivery through the OTM route as substantiated by novel formulations that have recently been marketed. Clinical studies have shown the safety and efficacy of these dosage forms, which many times exceeds or meets the performance of alternate dosage forms. The rapid onset of action, simplicity, and discreetness of administration has led to greater patient preference of these formulations and consequently greater patient compliance.

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Chapter 9
Formulation of Delivery Systems for Photosensitisers Used in Oral Cavity Photodynamic Therapy

Ryan F. Donnelly

9.1 Introduction

Photodynamic therapy (PDT) is a clinical treatment that combines the effects of visible light irradiation with subsequent biochemical events that arise from the presence of a photosensitising drug (possessing no dark toxicity) to cause destruction of selected cells [1]. The photosensitiser, when introduced into the body, accumulates in the target cells and a measured light dose of appropriate wavelength is then used to irradiate the target tissue [2, 3]. This activates the drug through a series of electronic excitations and elicits a series of cytotoxic reactions, which can be dependent on, or independent of, the generation of reactive oxygen species [4].

PDT has progressed considerably from the early application of sunlight and haematoporphyrin derivative, to the use of Photofrin®, and to second-generation-preformed photosensitisers and topical (surface) application of the prodrug, 5-aminolevulinic acid (ALA) which leads to in situ synthesis of protoporphyrin IX (PpIX) [5]. Topical PDT is now used for a variety of malignant, dysplastic, hyperplastic and infectious skin disorders [6, 7]. Clinical acceptance of topical PDT, in particular, has been accredited to the pioneering work of Kennedy et al. [8]. The results of this first clinical trial of topical PDT exploited the tumour-selective accumulation of the photosensitiser, PpIX, following topical cutaneous application of ALA. A 90% clearance rate was achieved in 80 lesions treated with 20% weight for weight (w/w) ALA in an oil-in-water (o/w) cream followed, 3–6 h later, by local illumination from a 500-W lamp equipped with a 600-nm-long-wave pass filter. The popularity of ALA, as the most commonly studied agent for PDT, is clearly evident in the number of published articles on the topic, which has increased markedly from 2 in 1991 to about 13,000 in 2013 [9].

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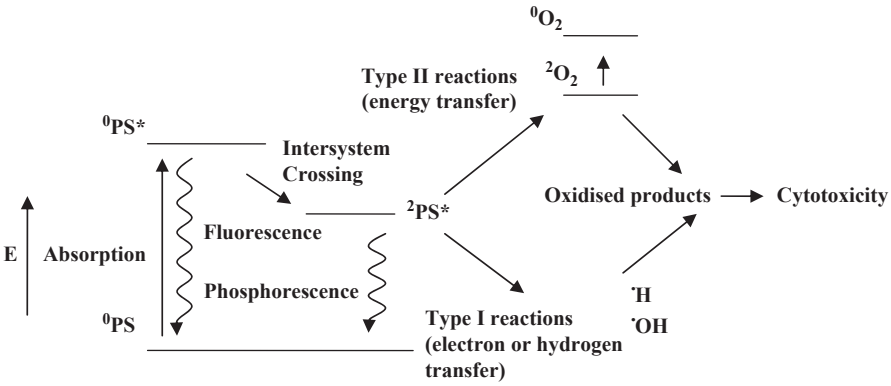

Fig. 9.1 The mechanism of action of photodynamic therapy (PDT). Numbers in superscripts denote the number of unpaired electron spins in each molecule

The detailed mechanism of action of PDT has been discussed extensively elsewhere [10–12]. Briefly, it results from the interaction of photons of visible light, of appropriate wavelength, with intracellular concentrations of photosensitising molecules (Fig 9.1). Photosensitisers have a stable electronic configuration, which is in a singlet state in their lowest or ground energy level [11]. This means that there are no unpaired electron spins [13, 14]. Following absorption of a photon of light of specific wavelength, a molecule is promoted to an excited state, which is also a singlet state and is short lived with a half-life between 10^{-6} and 10^{-9} s [11, 12]. The photosensitiser can return to the ground state by emitting a photon as light energy, or, in other words, by fluorescence, or by internal conversion with energy lost as heat. Alternatively, the molecule may convert to the triplet state. This conversion occurs via intersystem crossing which involves a change in the spin of an electron [15]. The triplet-state photosensitiser has lower energy than the singlet state but has a longer lifetime.

The singlet-state sensitiser can interact with surrounding molecules via Type I reactions, while the triplet-state sensitiser can interact with its surroundings via Type II reactions. The former type of reaction leads to the production of free radicals or radical ions, via hydrogen or electron transfer. These reactive species, after interaction with oxygen, can produce highly reactive oxygen species, such as the superoxide and peroxide anions, which then attack cellular targets [10]. However, Type I reactions do not necessarily require oxygen and can cause cellular damage directly, through the action of free radicals, which may include sensitiser radicals. Type II reactions, by contrast, require an energy-transfer mechanism from the triplet-state sensitiser to molecular oxygen, which itself normally occupies the triplet ground state [3]. Although possessing a short lifetime of approximately 10^{-6} s, a sufficient concentration of highly cytotoxic singlet oxygen is produced to induce irreversible cell damage [10, 11]. In addition, the photosensitiser is not necessarily destroyed, but can return to its ground state by phosphorescence without chemical alteration and may be able to repeat the process of energy transfer many times [15]. Alterna-

tively, the sensitiser may return to ground by transferring its energy to molecular oxygen, and may even be destroyed by photobleaching due to oxidation [16]. Evidently, many effects of PDT are oxygen dependent and rely on the oxygen tension within the target tissue. Types I and II reactions can occur simultaneously, and the ratio between the two depend on the photosensitiser, substrate, oxygen concentration and sensitiser to substrate binding [10]. Singlet oxygen is, however, widely believed to be the major damaging species in PDT [1, 2, 11]. Due to its extreme reactivity, singlet oxygen has a short lifespan in a cellular environment and limited diffusivity in tissue, allowing it to travel only approximately 0.1 μm [17]. This, combined with the facts that normal tissue may not contain photosensitiser or may not be perfused by blood vessels damaged by PDT, mean that normal tissue is normally unaffected by exposure to light [2].

9.2 Photosensitisers

The efficacy of certain types of dye against microbial species formed the basis of modern chemotherapy more than 100 years ago. The selectivity, particularly of cationic dyes, for bacteria over mammalian cells was used by Ehrlich and Browning to develop early synthetic antibacterials. However, much of the impetus for this work was lost at the inception of the antibiotic era, when the action of penicillin was seen as miraculous. The recent renaissance in the use of dyes and their derivatives in cancer treatment (PDT) relies on the fact that the dyes act as photosensitisers.

In order for a molecule to act as an efficient photosensitiser, it must possess the ability to absorb visible light, becoming excited to the triplet state, and then transfer its energy economically to molecular oxygen. Molecules possessing such characteristics are typically rigid planar structures possessing a high degree of conjugation. The major photosensitiser classes employed to date in photodynamic antimicrobial chemotherapy (PACT) include the porphyrins, the phthalocyanines and the phenothiaziniums (Fig. 9.2). The phenothiaziniums have simple tricyclic planar structures, typically cationic in nature. The most widely used compounds are methylene blue (MB) and toluidine blue (TBO). Both are efficient producers of singlet oxygen and the maximum absorption wavelength in water is 656 nm for MB and 625 nm for TBO, respectively. The porphyrins are heterocyclic *macrocycles* derived from four *pyrrole*-like subunits interconnected via their α carbon atoms via *methine* bridges. The absorption spectrum of porphyrins exhibits a maximum in the Soret band in the visible region of the electromagnetic spectrum between 360 and 400 nm, followed by four smaller peaks between 500 and 635 nm (Q-bands) [18]. The pyrrole groups in phthalocyanines are conjugated to benzene rings and bridges by aza nitrogens rather than methane carbons. This causes the absorption spectrum to shift to longer wavelengths and the Q-bands to become more intense than the Soret peak [19].

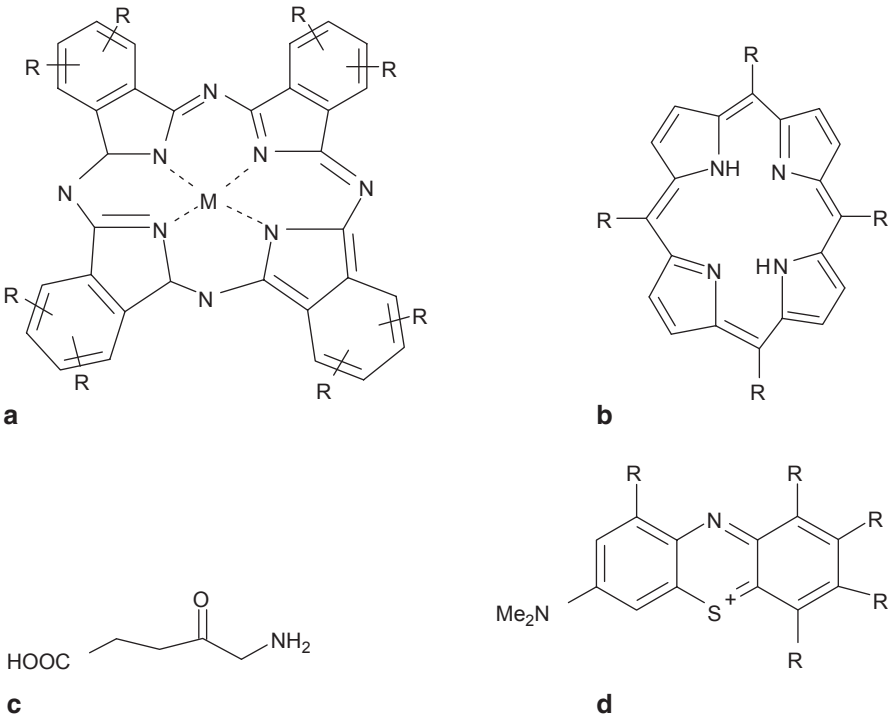

Fig. 9.2 Basic chemical structures of phthalocyanine (**a**), porphyrin (**b**), 5-aminolevulinic acid (ALA) (**c**) and phenothiazinium (**d**) photosensitisers investigated for potential use in photodynamic therapy (PDT) of nail diseases

9.3 Localisation of Photosensitisers

ALA is a small, water-soluble prodrug that is a naturally occurring precursor in the biosynthetic pathway of haem. The administration of excess exogenous ALA avoids the negative feedback control that haem exerts over its biosynthetic pathway. Due to the limited capacity of ferrochelatase to convert PpIX into haem, the presence of excess exogenous ALA in cells induces accumulation of PpIX [8, 20, 21]. This effect is pronounced in sebaceous glands and also in neoplastic cells. It has been reported that certain types of neoplastic cells have not only reduced ferrochelatase activity but also enhanced porphobilinogen deaminase (PBGD) activity [3, 10, 22]. The localisation of preformed photosensitisers is, however, not completely understood. Various theories exist regarding the preferential uptake by and accumulation of such agents in tumours. New photosensitisers, exhibiting rapid maximal accumulation in tumours, high tumour to normal tissue ratios and efficient clearance from the body are being actively sought [23, 24].

Preformed, lipophilic sensitisers, such as the porphyrins and phthalocyanines, when administered intravenously, are believed to be transported in the bloodstream

bound to lipoproteins such as low-density lipoproteins (LDLs) [25, 26]. Tumour cell membranes have been shown to possess disproportionately high numbers of LDL receptors [27] leading to large numbers of sensitiser molecules being brought into intimate contact with the tumour cells. Following receptor-mediated endocytosis, the sensitiser molecules may preferentially accumulate in the lipophilic compartments of the cells, including plasma, mitochondrial, endoplasmic reticulum, nuclear and lysosomal membranes [25]. This rather simplistic view does not provide the whole picture, however. In fact, in in vitro tissue culture experiments, tumour cells do not take up any more sensitiser than normal cells [12]. The in vivo situation is significantly different. Due to the rapidly growing nature of tumours with respect to normal tissue, their microvasculature is substantially altered, meaning they have a disordered blood supply and are less well perfused [1, 2]. They exhibit an enhanced vascular permeability to plasma proteins, show poor lymphatic drainage and have a larger interstitial space. The net result is that sensitisers exhibit enhanced transport to and prolonged residence in tumours. Bound sensitiser, accumulated in the tumour, can then be taken up by the cell as described above [1, 2, 12].

The uptake and retention mechanisms for free sensitisers located in the interstitial space or tumour microvasculature are distinct from those of bound sensitisers. As a result of the reduced tumour perfusion, tumour cells are forced to undergo anaerobic glycolysis, producing large quantities of lactic acid. Hydrolysis of adenosine triphosphate also occurs [28]. This means that the tumour interstitial pH is significantly lower than that of normal tissue [29]—an average pH value for tumours of pH 6.5 compared to approximately pH 7.5 for normal tissues not being unusual [12, 29]. Many photosensitisers are weak acids and, at low pH, will be largely unionised. Therefore, their cellular absorption will be enhanced by the lowered pH in the tumour microenvironment. Indeed, if tumour pH can be further lowered by administration of agents, such as glucose [30], an increased proportion of tumour cells may be killed directly by PDT [31]. Once the sensitiser molecule is within the cell, the higher intracellular pH, which is close to normal intracellular pH of around pH 6.9, may increase the proportion of ionised sensitiser. This ionised species then becomes temporarily trapped within the cell until such time as the extracellular concentration of sensitiser falls and the complex system of ionic equilibria which exists allows it to diffuse out of the cell as a neutral molecule. This latter principle also applies to sensitiser entering the cell by other means. Hence, there exists a defined time frame for each lipophilic sensitiser and type of tumour between sensitiser administration and its maximal accumulation within tumour cells [12].

Preformed hydrophilic sensitisers, such as water-soluble phthalocyanines, are largely carried by albumin and other serum proteins after intravenous injection [15]. These sensitisers then accumulate within the interstitial space and the vascular stroma of tumours due to their enhanced vascular permeability to plasma proteins, poor lymphatic drainage, and larger interstitial space [12]. Due to their low lipophilicity, these sensitisers do not readily diffuse across cellular membranes, although a small fraction may be absorbed by pinocytosis or endocytosis [12, 15]. As with lipophilic sensitisers, there exists an optimum time period between administration

of hydrophilic sensitisers and their maximal accumulation within tumours. Again, this time period will vary between different sensitisers and tumour types.

Tumours are not the only type of tissue which exhibits accumulation of photosensitisers. For example, the accumulation of certain sensitisers by the rapidly developing retinal neovasculature which is characteristic of age-related macular degeneration and the plaques of psoriasis has been used to achieve positive therapeutic outcomes by several workers [32, 33]. In addition, several normal body tissues high in reticuloendothelial components, such as the liver, exhibit accumulation of administered photosensitisers. This is a phenomenon which is not well understood.

It is now well known that cationic photosensitisers are more efficient than their neutral or anionic counterparts in the photodynamic killing of microbial cells. Cationic photosensitisers are more effective, especially as broad-spectrum antibacterials, than their anionic counterparts [34], as shown by their greater activity against Gram-negative bacteria, which have a more complex structure due to the presence of an outer membrane. The cell envelope of Gram-negative bacteria consists of an inner cytoplasmic membrane and an outer membrane that are separated by the peptidoglycan-containing periplasm. The outer membrane, which is highly negatively charged, forms a physical and functional barrier between the cell and its environment. It has been shown that anionic and neutral photosensitisers can become effective against Gram-negative bacteria when coadministered with a cationic agent, such as polymyxin [35]. However, for simplicity and because, even against more susceptible Gram-positive bacteria, cationic photosensitisers appear to be more effective [34, 35], these cationic agents are the predominant type used in PACT. To date, there have been several reports on the use of photosensitisers and light to kill both yeasts and other fungi. However, there has been much less systematic study on the types of physicochemical properties necessary in a photosensitiser in order to make it effective in mediating photodynamic killing of such microorganisms. Fungi present much more complex targets than bacteria. For example, yeasts, which constitute a large group of rather disparate eukaryotic organisms, are enveloped by a thick external wall composed of a mixture of glucan, mannan, chitin and lipoproteins and separated from the plasma membrane by a periplasmic space. However, the available evidence suggests that the response of such cells to photodynamic processes is less strictly controlled by structural factors as compared with bacteria [34, 35]. Nevertheless, similarities with mammalian cells should be considered, and this may indicate the use of cationic photosensitisers, rather than their anionic counterparts, since the latter exhibit facile uptake by mammalian cells [19].

Uptake of exogenous substances by fungi is generally adversely affected by lipophilicity and positively affected by hydrophilicity and the presence of charged groups. Following uptake, photosensitisers are distributed to subcellular targets. The pattern of localisation is important, as targets adjacent to the photosensitiser have the greatest probability of being involved in photodynamic processes, due to the high reactivity and short lifetime of the singlet oxygen generated. The biochemical and functional effects of photosensitisation include inactivation of enzymes and other proteins and peroxidation of lipids, leading to the lysis of cell membranes, lysosomes and mitochondria [34, 35]. Thus, singlet oxygen generated by excitation

of photosensitisers is a nonspecific oxidising agent. Consequently, there is no cellular defence against it. Indeed, antioxidant enzymes, such as catalase and superoxide dismutase are inactivated by it. This means that there should be no difference in susceptibility to PACT between organisms resistant to conventional antifungals and their naïve counterparts. The high reactivity of singlet oxygen has other advantages, because even though the localisation of the photosensitiser may be determined by its physicochemical properties, the diffusion of singlet oxygen should be sufficient to be able to inactivate other structures and biomolecules. Therefore, it is unlikely that fungi could readily evolve resistance to singlet oxygen. In addition, photodynamic processes have never been associated with mutagenic effects in microorganisms. Moreover, singlet oxygen is only present during illumination and fungi are not continuously exposed to it, as they are with conventional antifungals. Furthermore, singlet oxygen cannot travel to other sites in the body, such as the intestinal tract, during treatment. These latter facts make development of resistance even more unlikely.

It has been widely noted that *Candida albicans,* like other yeasts is slightly more difficult to kill by PACT than Gram-positive bacterial cells, necessitating higher drug and light doses [34, 35]. This has been attributed to the presence in the yeasts of a nuclear membrane, the greater cell size and the reduced number of targets for singlet oxygen per unit volume of cell [36]. However, it has been shown that the photosensitiser and light doses producing high levels of kill in yeasts in vitro do not kill appreciable numbers of human cells under the same conditions and cause no detectable genotoxic or mutagenic effects [19, 34–36]. Should photodynamic killing of fungi be carried out in vivo, then the limited diffusion distance of singlet oxygen from its site of generation and the fact that illumination would be limited to the area of infection means that selectivity for fungi over host cells would be further enhanced.

9.4 Light Administration

By definition, PDT requires a source of light to supply the requisite energy for singlet oxygen production in situ. The energy required is determined by the molecular structure of the photosensitiser and, thus, a different light excitation range is required for the phenothiaziniums (ca. 600–660 nm) than for the phthalocyanines (ca. 630–690 nm). Ideally, light sources should provide a strong output at the requisite wavelength for photoexcitation. Lasers, and the less expensive and easier to use, filtered incoherent lamps are the most commonly employed sources in PACT today. White or fluorescent light sources may be used. However, for in vivo use, emission in the ultraviolet range should be minimised, due to the risk of mutagenesis. Similarly, emission in the infrared range is also undesirable, so as to avoid heating of tissue. Typical power outputs for light sources used in antifungal PACT are in the range 10–100 mW cm^{-2}, with typical total light doses being between 10 and

200 J cm^{-2}. In some cases, these may need to be higher than those used in antibacterial PACT in order to yield comparable rates of kill [37].

Light influence through tissue decreases exponentially with thickness. This decrease is determined by absorption, particularly by haemoglobin, and scattering, parameters that vary between tissue types [38]. Due to the inability of light to penetrate deeply into tissue, clinical PACT is necessarily limited to areas of the body that can be irradiated from the surface. Thus, antifungal treatment would be restricted to infections of the skin, nails, hair, oral cavity, oesophagus, and lower female reproductive tract. In treating such infections, however, some degree of tissue penetration is required, for example, to kill fungi residing below the surface of the skin or in the matrix of the nail. Light in the red region of the spectrum penetrates tissue down to around 3.0 mm, while light in the blue region penetrates down to only around 1.5 mm. Thus, the porphyrins are typically excited by light in the red region of the spectrum, rather than blue light, which they absorb more efficiently [39]. Consequently, much work has been devoted to the phthalocyanines, which absorb more effectively at longer wavelengths.

Endogenous light absorption is important in clinical applications of antifungal PACT. It is essential that photosensitisers used to kill fungi can be photoexcited, and this will not occur if the incident light is absorbed by fungal pigment. Thus, photosensitisers absorbing beyond the range of the pigment are required, with appropriate light sources. As with all proposed protocols, a thorough knowledge of the photoproperties of both target and agent will be essential. It is also vitally important that light can penetrate efficiently through tissue to reach the site of infection.

9.5 Delivery of Photosensitisers

Many skin tumours are treated by PDT following intravenous injection of sophisticated formulations of preformed photosensitiser. Oral lesions and infections, which tend to be more superficial in nature, are typically now treated by topical application of a photosensitiser-containing vehicle prior to visible light irradiation. However, despite the vast number of studies published in the area of topical PDT of oral lesions and infections, a rational approach to formulation design has not taken place. The treatment is still considered as a largely experimental approach, often being used if a patient refuses conventional treatments, such as chemotherapies. This may be because this field is dominated by clinicians and basic scientists, rather than those involved in pharmaceutical formulation development. When formulating a topical drug delivery system, the aim should be to maximise the thermodynamic activity of the drug substance in the vehicle, so as to maximise the concentration drive for diffusion and the partition coefficient between the oral mucosa and vehicle. For example, formulating a relatively lipophilic ALA derivative, such as the hexyl ester in an aqueous vehicle should maximise its flux into lesions of the oral cavity when applied topically. Many oral conditions have been treated using PDT, with a variety of different formulation approaches taken. These are discussed in detail below.

9.5.1 Oral/Buccal Drug Delivery Systems for Neoplastic Diseases

Various cancerous, precancerous and dysplastic lesions of the oral mucosa and tongue have been treated clinically with PDT over the past 25 years. Initially, the only commercially available formulation, Photofrin® II, was injected intravenously and the oral lesions then irradiated subsequently. As early as 1988, Schweitzer and colleagues treated five patients with oral Kaposi's sarcoma (the most common malignancy in patients with acquired immune deficiency syndrome) and achieved a complete or partial response in all five [40]. Haematoporphyrin derivative (HpD, Photofrin® II), at a dose of 2 mg kg^{-1} intravenously, was the sensitiser. The light dose, administered 48 h after the sensitiser from a laser source, varied from 30 to 400 J cm^{-2}. Hebeda et al. [41] reported excellent initial response rates, which were between 50 and 100%, for cutaneous and oral Kaposi's sarcoma. The median duration of response was 3 months. A similar HpD and light-dosing regimen as the other study was used. Lesions of the mouth, head and neck responded best, while the lesions of the trunk and extremities, particularly nodular or hyperpigmented lesions, did not respond as well. However, in contrast to the other study, severe pain and scarring were noted as side effects. The authors suggested that, while excellent cosmetic results were obtained by other investigators using PDT, the severe scarring seen here could be explained by the differences between the lesion types. While conditions such as Bowen's disease were usually confined to the epidermis, Kaposi's sarcoma often extended beyond the mucosa to the tissue beneath. After PDT-induced tumour destruction, the resulting, much deeper, wounds impair healing and lead to scar formation. The immunocompromised, often debilitated nature of Kaposi's sarcoma patient exacerbates the situation.

More recently, clinicians have favoured topical application of photosensitisers or their precursors so as to avoid widespread prolonged cutaneous photosensitisation. Typically, an aqueous solution is placed in the patient's mouth or a cream normally intended for topical application to the skin is employed. Following a suitable interval, light irradiation is performed. Kvaal et al. [42] applied Metvix cream (16% w/w ALA–methyl ester) to lesions of oral lichen planus (chronic mucocutaneous disease that affects the skin, tongue and oral mucosa) in 14 patients followed by red-light irradiation. As a result of one treatment session, there was a significant improvement of oral lichen planus after 6 months and during a 4-year follow-up period. Twenty patients with symptomatic oral lichen planus were treated by Sadaksharam et al. [43]. They were treated with 5% w/v MB solution-mediated PDT (light source: Xenon arc lamp, wavelength: 630 ± 5 nm, total dose: 120 J/cm^2 per sitting) in four sessions (1st, 4th, 7th and 15th day). Follow-up was done on the 2nd and 4th week after the therapy. There was significant improvement in signs and symptoms of the lesion at first and second follow-up visits. The investigators concluded that there was satisfactory reduction in signs and symptoms of oral lichen planus without any side effects. Thus, MB-mediated PDT seems to be a promising alternative for the control of oral lichen planus.

Kawczyk-Krupka et al. [44] aimed to compare the curative effects of PDT and cryotherapy in the treatment of oral leukoplakia (a premalignant lesion of the oral mucosa). The first patient group, treated by topical PDT (ALA, 630–635 nm wavelength), consisted of 48 patients suffering from leukoplakia. The second group consisted of 37 patients treated using cryotherapy. Analyses and comparisons of the complete responses, recurrences, numbers of procedures and adverse effects after both PDT and cryotherapy were obtained. In the first group, a complete response was obtained in 35 patients (72.9%), with 13 recurrences observed (27.1%) over a 6-month period. In the second group, a complete response was obtained in 33 patients (89.2%), and recurrence was observed in 9 patients (24.3%). PDT and cryotherapy appear to be comparative methods of treatment that may both serve as alternatives for the traditional surgical treatment of oral leukoplakia. The authors stated that the advantages of PDT are connected with the minimally invasive and localised character of the treatment and the lack of damage to collagenous tissue structures. They concluded that PDT was more convenient for patients, was less painful and promoted tissue preservation.

A range of other studies have been conducted. However, no sophisticated drug delivery systems have been described, with simple solutions or topical semisolids the mainstay of treatment. One notable exception was the work of Donnelly et al. [45], who described a bioadhesive patch that was capable of remaining in place on the lip for prolonged periods of time and delivering ALA for use in photodiagnosis. Clearly, additional work is required in this area to enhance the success of PDT on neoplastic and dysplastic lesions of the oral cavity.

9.5.2 Oral/Buccal Drug Delivery Systems for Infectious Diseases

If the delivery of photosensitiser was optimised, PACT could prove a viable alternative treatment regimen for oral candidiasis. A study carried out by Teichert et al. investigated the potential of MB-mediated PACT in the treatment of oral candidiasis in an immunosupressed murine model, mimicking the conditions in an immunodeficient human [46]. Complete eradication of *C. albicans* infection was achieved after MB-PACT. In the study, the mice were inoculated with *C. albicans*-coated swabs on a thrice weekly basis for 4 weeks prior to the experiment, with drinking water also inoculated. At the beginning of the PACT experiment, mice were swabbed to confirm infection status, before instillation of 0.05 ml of aqueous MB solution in concentrations ranging between 250 and 500 μg ml^{-1}. After 10-min incubation, the oral cavity of the mice was irradiated with a diode laser light coupled with a 1.0-cm cylindrical diffuser, emitting at 664 nm and delivering a total light dose of 275 J cm^{-2}, with culturing taking place immediately after irradiation. Complete eradication was noted after 450 and 500 μg ml^{-1} MB-mediated PACT, with lower MB concentrations associated with a reduction in colony-forming units isolated, when compared to negative control and pretreatment samples. Application of a photosensitiser solution would cause staining of the buccal cavity and teeth, which

would be cosmetically unacceptable in human therapy. Therefore, development of a more aesthetically pleasing method of photosensitiser delivery is important before the therapy could be used clinically.

Donnelly et al. formulated a patch containing TBO, a phenothiazinium compound structurally similar to MB, for treatment of oral candidiasis [37]. The patch was identical to that containing ALA [45] with TBO, at a drug loading between 10 and 100 mg cm^{-2}, simply replacing the ALA. The patches were capable of resisting dissolution when immersed in artificial saliva. When releasing directly into an aqueous sink, patches containing 50- and 100-mg TBO cm^{-2} both generated receiver compartment concentrations exceeding the concentration (2.0–5.0 mg ml^{-1}) required to produce high levels of kill (> 90 %) of both planktonic and biofilm-grown *C. albicans* upon illumination. However, the concentrations of TBO in the receiver compartments separated from patches by membranes intended to mimic biofilm structures were an order of magnitude below those inducing high levels of kill, even after a 6-h release. Therefore, the authors suggested that short application times of TBO-containing mucoadhesive patches should allow treatment of recently acquired oropharyngeal candidiasis, caused solely by planktonic cells. Longer patch application times may be required for persistent disease where biofilms are implicated.

Lin et al. [47] showed that TBO-mediated PACT (red light, 48 J cm^{-2}) eradicated 97 % of microorganisms from oral wound infections in rats. Wound size post-PACT was also significantly reduced compared to controls, further emphasising the potential of pheothiazinium-based PACT in the treatment of oral infections.

Many studies have also reported the suitability of PACT for the treatment of periodontitis, which is an inflammatory disease caused by bacteria and affects the gums, bone and other supporting tissues of the teeth [48]. Pathogens isolated from patients with periodontal disease have been successfully killed by PACT. For example, TBO-mediated PACT has been determined, in vitro, to be lethal to planktonic and biofilm cultures of *Streptococcus sanguis, Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* under specific conditions [49]. Photosensitiser conjugates have also been used to induce photodynamic killing in several pathogens involved in periodontal disease [50]. The resulting increased specificity of photosensitiser and potential narrowing spectrum of activity could be an issue in clinical therapy where, as mentioned previously, it would be of benefit for a photosensitiser to be active against a multitude of oral pathogens. Bhatti et al. demonstrated that the presence of serum adversely affected the killing ability of TBO–PACT over *P. gingivalis* in vitro [51]. In vitro PACT treatment of plaque biofilms formed in vivo determined that the thickness of biofilms could be halved after pyridinium Zn (II) phthalocyanine-mediated PACT [52].

Kömerik et al. inoculated the oral cavities of rats with a 25-µl suspension of *P. gingivalis,* corresponding to 10^{10} colony-forming units per ml of bacteria [53]. The oral cavity was treated either immediately after inoculation or after disease development, by exposure to 25 µl TBO in 0.85 % w/v NaCl, at a concentration between 0.01 and 1.0 mg ml^{-1}, followed by exposure to 630-nm laser light from a fibre optic cable at a total dose between 6 and 48 J. In all cases, almost 100 % kill was

achieved. Where the disease was allowed to progress before treatment, treated rats had markedly reduced bone loss in comparison with controls.

A similar study completed by Sigusch et al. used a beagle dog model to determine in vivo susceptibility of oral periodontal pathogens to PACT mediated by two photosensitisers chlorin$_{e6}$ and BLC 1010 [54]. Animals were inoculated with *P. gingivalis* and *F. nucleatum* and, 4 weeks later, when infection was established, a non-disclosed quantity of 10 μM photosensitiser, dissolved in PBS, was applied to the sites of infection. An optical fibre was used to deliver light from a diode laser, emitting at 662 nm and delivering a total light dose of 12.7 J cm^{-2} to each infection site. Treatment resulted in a significant reduction in both periodontal inflammation and bacterial load.

The method of drug delivery in both studies is simplistic and would not be aesthetically acceptable in human patients. Photosensitisers are highly coloured compounds, and so their delivery to the site of infection should be targeted. One such drug-delivery device is the Periowave® system. Currently, the system allows targeted delivery of MB—a treatment option in Canada for patients with periodontitis. The treatment kit contains 0.005 % w/v MB in phosphate-buffered saline, and hydroxymethylcellulose as a mucoadhesive [55]. The solution is delivered via an irrigating needle. This system is targeted, in that the needle allows mechanical targeting of the solution to the desired site. After irrigation of the periodontal pocket, a fibre optic tube is used to deliver a total light dose (670 nm) of between 10 and 20 J cm^{-2} over a 60-s period. Use of the Periowave® system in combination with scaling and root planing was found by Andersen et al. to increase the clinical attachment level (CAL) of the gum by threefold in comparison to scaling and root planing alone. Another system, the SaveDent® light-delivery system, is used in conjunction with a solution of pharmaceutical-grade TBO, both provided by Denfotex Ltd, a UK-based company [56]. Again, the treatment area, in this case a root canal, is irrigated with an aqueous solution of the photosensitiser at a concentration of 12.7 μg ml^{-1} using an endodontic micro-needle and, after a 60-s incubation period, is irradiated with the SaveDent® device for 60 s at 100 mW. In an in vivo study, the Savedent® system led to culture-free canals in 93 % of cases, compared to 76 % of patients whose root canals were disinfected by conventional methods, namely irrigation with 2.25 % aqueous sodium hypochlorite solutions.

Thermoresponsive hydrogels composed of *N*-isopropylacrylamide and hydroxyethylmethacrylate loaded with zinc tetraphenylporphyrin have been found, by Jones et al. to demonstrate drug release properties making them suitable for implant devices [57]. To prepare the gel, the required masses of monomer (2 g in total), namely *N*-isopropylacrylamide (NIPAA) or mixtures of NIPAA and 2-hydroxyethyl methacrylate (HEMA) were dissolved in 8 g of deionised water with stirring, after which, 12.5 mg of potassium persulphate and 0.125 ml *N,N',N'',N*-tetramethylethylenediamine (TEMED) were added and stirred until dissolution had occurred. Polymerisation was then allowed to occur for 16 h at 20 °C, and when required, zinc tetraphenylporphyrin (Zn-TPP, 0.04 % w/w) was dissolved in the monomer solution prior to polymerisation. Studies on the gel consisted of characterisation of mechanical properties such as hardness and compressibility of the hydrogel and drug

release. To determine drug release, sections of the various hydrogels, contained within a 10-cm^3 circular mould, were immersed into and anchored to the surface of beakers containing deionised water at either 20 or 37 °C and the beakers incubated at 20 or 37 °C in a water bath, shaking at 100 oscillations min^{-1}. The release of the photosensitiser was dependent on the formulation of the gel and on temperature. For example, the time required for the release of 15 % w/w of the original loading of drug from *p*(HEMA) at 20 °C was 35.75 min, whereas for the *p*(NIPAA-*co*-HEMA) hydrogels this value increased to approximately 47 min. The release of the photosensitiser may be controlled by heating or cooling of the gel, which swells in response to reduction of temperature and releases drug.

Possible uses suggested by the author for this system include the treatment of periodontal disease or infected wounds. The hydrogel may be syringed into the periodontal pocket, where, at the temperature within the buccal cavity, no drug would be released. Reduction of temperature by 3 °C by irrigation with cold water would cause photosensitiser release. The released photosensitiser could then be irradiated using a fibre optic tube. One of the benefits of using this hydrogel is that, once the photosensitiser is released from the matrix, the hydrogel could be reloaded by immersion in photosensitiser solution at a temperature below the lower critical solution temperature. This novel method of controlled drug delivery could be useful in this arena, although extensive work is required before hydrogels could be used routinely in the clinical situation.

Lulic et al. [58] have recently reported the outcome of a 1-year follow-up study where ten patients with periodontitis were treated with PACT five times over a 2-week period. They found greater reductions in the depth of periodontal pockets in PACT-treated patients than in control patients, which is indicative of healing. The use of PACT in treatment of periodontitis is now gaining significant momentum, with treatment shown to reduce bacterial load in periodontal pockets, inactivate bacterial virulence factors and host cytokines that impair periodontal restoration. Furthermore, there is minimal chance of resistance development, and the adjacent host tissue and microflora are not adversely affected.

9.6 Conclusion

It is clear that PDT has an important role to play in the treatment of neoplastic and dysplastic disease at body sites amenable to irradiation, including the oral cavity. While systemic delivery systems for photosensitising drugs have reached a high level of sophistication, the same cannot be said for topical delivery vehicles, where much work is still required in order to enhance tissue penetration and improve therapeutic outcomes for patients, especially those suffering from deeper lesions. Studies published to date on topical application of photosensitisers and photosensitiser precursors have used aqueous solutions, oil in water creams, water in oil creams, hydrogels, organogels, sponge and cubic phase formulations and aqueous- and solvent-based patches. These dosage forms, which in many cases seem to have

been selected at random with little regard to their nature, possess a multitude of different physicochemical properties. This has made comparison of different studies difficult. As a result, the true value of derivatisation of ALA to yield more lipophilic prodrugs, for example, has been blurred somewhat. In addition, the arbitrary dosing approach taken to topical application of ALA has caused further confusion. Mucoadhesive patches, which could overcome this latter problem, have not yet gained acceptability from clinicians and so remain as experimental formulations. Topical application of preformed photosensitisers may offer considerable advantages over ALA and its derivatives in terms of reduced drug-to-light intervals and less pain on irradiation. However, their high molecular weights mean that sophisticated technologies, such as needle-free jet injections or microneedle arrays will have to be used to allow delivery to all but the most superficial of lesions.

In the foreseeable future, PDT of neoplastic and dysplastic lesions of the oral cavity is likely to continue to be based on topical application of simple semisolid dosage forms containing ALA or its methyl ester. Until expiry of patents on the current market leading products (Levulan® and Metvix®) approaches, there is unlikely to be a great incentive for pharmaceutical companies to engage in design and evaluation of innovative formulations for topical PDT. Consequently, such research will continue to be the preserve of academic departments, who rarely possess the funds for clinical trials. Ultimately, this may prove to be to the detriment of patients.

The situation with antimicrobial applications in the oral cavity is actually quite well developed. This is notable, since funding agencies currently see PACT as more of a curiosity than the viable therapy for antibiotic-resistant wound and burn infections that it is. That PACT has been commercialised for the clinical management of periodontal disease illustrates the effectiveness of the treatment. Management of candidiasis of the oral cavity will, however, require investment, since rinsing with a highly coloured photosensitiser solution prior to irradiation will not be acceptable for patients. A mucoadhesive patch may solve some of the compliance issues, once fully developed.

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Chapter 10
Medical Devices for Oral Mucosal Applications

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10.1 Definitions and Relevant Regulations

Medical devices have been the object of an extensive discussion within the European scenario over the last several years since the issuing of the new directives of the so-called *novel approach* [14, 15] that were launched by the European Commission (EC) and implemented in their national regulations. These directives arose due to the wide range of products belonging to the category of medical devices and the diversity of technical norms applied by the national bodies. The *novel approach* policy is aimed at the European harmonization of the minimum requirements to be fulfilled by the manufactures especially, but not exclusively, in terms of safety and effectiveness, and taking into account the intended purposes of the concerned devices.

Even though the regulatory approach adopted overseas, in the US scenario, is different from the European one, the regulatory bodies of both regions are similarly concerned with the levels of control needed to assure safety and effectiveness of the device.

Instead of giving a punctual definition, Food and Drug Administration (FDA) classification encompasses a variety of products that belong to the category stating that 'medical devices range from simple tongue depressors and bedpans to complex programmable pacemakers with micro-chip technology and laser surgical devices. In addition, medical devices include in vitro diagnostic products, such as general purpose lab equipment, reagents and test kits, which may include monoclonal antibody technology. Certain electronic radiation emitting products with medical

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application and claims meet the definition of medical device. Examples include diagnostic ultrasound products, x-ray machines and medical lasers' [19].

According to the more punctual CE definition 'A medical device means any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, together with any accessories, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of:

- Diagnosis, prevention, monitoring, treatment or alleviation of disease
- Diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap
- Investigation, replacement or modification of the anatomy or of a physiological process
- Control of conception,

and which does NOT achieve its principal intended action IN or ON the human body by pharmacological, immunological or metabolic means, but which may be assisted in its functions by such means' [14].

The CE definition attempts to answer the main questions related to medical devices, in particular: What is it? Why use it? How does it *not* work? The answer to the third question is especially relevant since it is functional to the delineation between medical devices and pharmaceuticals. This in turn requires an answer to two basic questions: what is the principal intended action, as assigned by the manufacturer, and how is this principal intended action achieved? To be considered a medical device, even though a therapeutic purpose is pursued, the main mechanism of action should not be a pharmacological, an immunological or a metabolic one. In particular, if a medicinal substance is incorporated to assist and complement the principal action of the device, the ancillary nature of the additional medicinal substance must be clearly established. As it will become clear in the following section, to be able to answer this question enables the correct design of a product. The classification of medical devices is also functional to a correct development of any product, since the amount of documentation to be provided will depend on the class.

In both regions, the classification of medical devices depends on the intended use of the device and also on the indications for use, but is mainly risk-based, meaning that the risk that the device poses to the patient and/or the user is a major factor to be considered. In both regulations the extent of attention of the regulators is proportional to the level of risk related to the use of the specific product.

According to the EC classification, medical devices are divided into four risk classes, from Class I (lowest risk), Class IIa and IIb (intermediate risk) and Class III (highest risk). Risk classification is based on: contact time, invasiveness (and therefore site of contact) and on whether the device is active or not. A scheme of EC classification is given in Fig. 10.1. Two basic principles, both risk-based, govern the EC classification and the regulation of medical devices: intrinsic safety and balance between risk and benefit. The devices must be designed and manufactured in such a way, that when used under the conditions and for the purpose intended, they will not

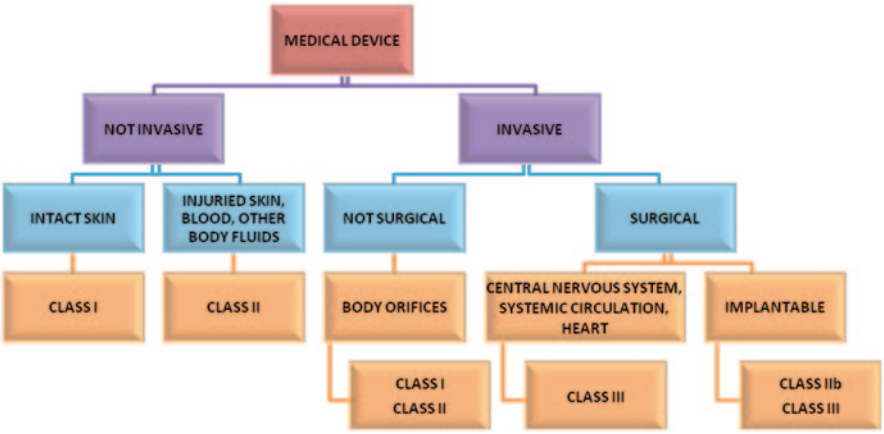

Fig.10.1 Classification scheme of Medical Devices according to EC

compromise the clinical conditions or safety of patients or any others, furthermore any undesirable side-effect must constitute an acceptable risk when weighed against the performances intended. Again the solutions adopted by the manufacturer for the design and construction must conform to safety principles, taking account of the generally acknowledged state of the art.

FDA has grouped the various types of devices into 16 medical specialties (panels) such as cardiovascular devices, ear, nose throat devices, etc. For each of the devices classified by the FDA, the Code of Federal Regulations (CFR) gives a general description including the intended use, the class to which the device belongs and information about marketing requirements. Device classification depends on the intended use of the device and also upon indications for use. In addition, classification into three regulatory classes (Class I, II and III) is risk-based, that is, the risk the device poses to the patient and/or the user is a major factor contributing to the class to which it is assigned. Class I includes devices with the lowest risk and Class III includes those with the greatest risk. Most importantly FDA classification determines the level of control necessary to assure the safety and effectiveness of the device and type of premarketing submission/application required for FDA clearance to market [19].

Examples of medical devices (as per EC classification) that may resemble more closely the delivery systems we are familiar with in the pharmaceutical field are:

- Bone cements containing antibiotic.
- Root canal fillers which incorporate medicinal substances with ancillary action.
- Soft tissue fillers incorporating local anaesthetics.
- Bone void filler intended for the repair of bone defects where the primary action of the device is a physical means as a scaffold for osteoconduction and where the ancillary nature of the eventual additional medicinal substance can be clearly established.

- Wound dressings, surgical or barrier drapes (including tulle dressings) with antimicrobial agent.
- Ophthalmic irrigation solutions principally intended for irrigation which contain components which support the metabolism of the endothelial cells of the cornea.
- Drug eluting coronary stents.

These examples are mainly representative and not exhaustive of a very broad category. The medical devices used in oral pathologies do not exactly belong to the above categories due to the characteristics of the technology involved; they are mainly liquid or semisolid forms (to be used as rinse, mouthwash or local applications) which resemble medicinal products. Besides that, the learned lesson from the above classification is that, when developing a product intended as a medical device, a clear representation of the principal mechanism(s) of action (that should not be either pharmacological or immunological or metabolic) envisaged for this particular product should be clearly stated and supported by a scientific rationale. It has also to be noted that the majority of medical devices that are described in this chapter belong to the Class IIa or IIb, meaning that they bear an intermediate level of risk [14, 15].

10.2 Oral Mucosa Conditions That Can Be Treated with a Medical Device

The oral cavity is the first part of the gastrointestinal tract and it represents a natural border between the external world and the inner part of the body. Oral mucosae (both keratinized and non-keratinized epithelial tissue), teeth, tongue, maxillary and mandibular muscles and salivary glands work all together in order to carry out several functions that are fundamental for the body's homeostasis (such as eating and drinking), as well as for social relationships (speech). All these activities are facilitated by the presence in the oral cavity of saliva, the product of salivary glands, which provides lubrication and hydration both of the hard and soft tissues present in the cavity. Moreover saliva contains enzymes (i.e. ptyalin) that initiate digestive activities in normal buccal conditions.

10.3 Oral Mucositis

The term 'mucositis' refers to inflammatory, erosive and ulcerative lesions of any part of the mucosa belonging to the gastrointestinal tract; in particular when this pathology is expressed in the oral cavity it is defined as oral mucositis [23]. Even though several etiologies are connected with the onset of mucositis (i.e. alimentary, allergic, immunological, etc.), mucositis, in particular oral mucositis, related to the chemo and radiotherapies used for cancer treatments, is the most frequent and predictable form of mucositis [33].

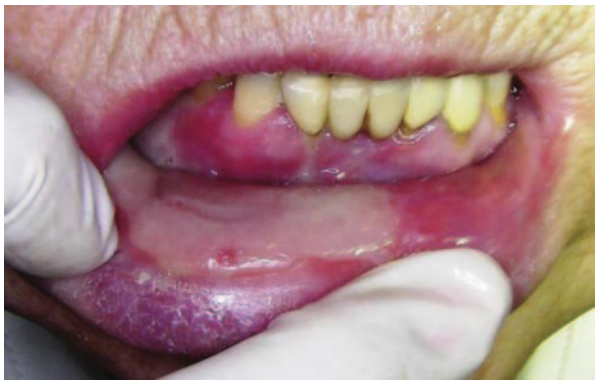

Fig. 10.2 Example of irradiation-induced oral mucositis

Recently the term ‘mucositis’ was shown to be associated to lesions of the gastrointestinal tract caused by cytotoxic cancer treatments with the address of the ICD-9 Code of 528.0, specific ICD-10 code of oral mucositis is K12.3 [43]. The incidence of oral mucositis was estimated in 10–40 % of cancer patients receiving treatment for solid tumours and up to 80 % in patients undergoing radiotherapy; the incidence is almost certain to occur in patients undergoing high-doses of chemotherapy as ablative treatment prior steam cell transplantation (89 %) [23].

10.3.1 Clinical Presentation

Oral mucositis is a progressive pathology, it initially presents as an erythema of the buccal mucosa with a reported feeling of burning, and the progression in a more severe stage is characterized by the insurgence of ulcerative lesions that, progressively, tend to be deeper and painful (Fig. 10.2). In Fig. 10.3, the phases of oral mucositis are schematically drawn. In general, the pain is so intense that an impossibility to eat and drink is manifested. According to the World Health Organization (WHO) classification, oral mucositis can be clinically classified over a five-point scale (Table 10.1). Other clinical research scales (e.g. Oral Mucositis Index—OMI; Oral Mucositis Assessment Scale—OMAS, reported in Table 10.2) are more sensitive but need more experience for the assessment [42]

10.3.2 Aphthous Stomatitis

Most of the recurrent aphthous ulcers are relatively mild solitary or multiple painful small (8–10 mm) lesions that occur at intervals of a few months in the oral cavity and spontaneously heal in 10–14 days. They are most commonly seen in the non-keratinized mucosal surfaces like labial mucosa, buccal mucosa and floor of the mouth. More serious but also less frequent types (Sutton’s disease) exceed

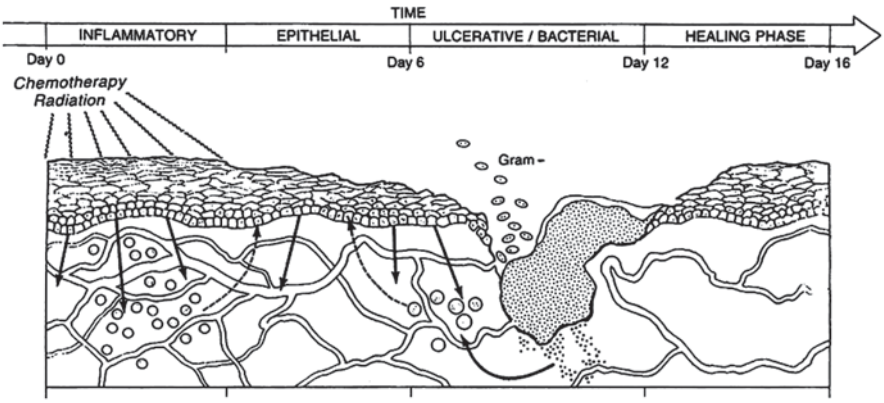

Fig. 10.3 Phases of oral mucositis [42] (with permission)

Table 10.1 WHO classification of mucositis grades

Severity	Oral mucositis			Severe oral mucositis	
Grades	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Clinical features	No change	Soreness/ erythema	Erythema and ulcers (diet with solids)	Ulcers (only liquid diet)	Alimentation not possible

Table 10.2 Oral mucositis assessment scale (OMAS)

Location			
Lip		Buccal mucosa	
	Upper		Right
	Lower		Left
Tongue ventrolateral		Palate	
	Right		Hard
	Left		Soft
	Floor of the mouth		
Ulceration grades			
0 = none	1 < 1 cm^2	2 = 1–3 cm^2	3 > 3 cm^2
Erythema grades			
0 = none	1 = not severe	2 severe	

1 cm in diameter, and persist for up to 6 weeks healing with scarring. Herpetiform ulceration is small in size but may be very numerous, up to 100 in number, and can coalesce into large irregular-shaped ulcers. Aphthous ulcers etiopathogenesis is still unclear in spite of their high diffusion. From the point of view of treatment, the strategies involve symptomatic relief by reducing pain and accelerating ulcer healing, so these lesions will be assimilated to mucositis [31].

Table 10.3 Mechanism of actions of the products/technique used in the symptom management of oral mucositis

Component	Function
Ice	Cryotherapy
Hyaluronic acid/PVP/glycirizzinic acid (Gelclair®), Carbomer homopolymer A (MuGard) Lecithin/glycerol dioleate (Episil) Sodium polystyrene sulfonate (Mucotrol)	Coating effect
Carbomer, acrylates, carboxymethylcellulose, chitosan, alginate, Tamarind gum, hyaluronic acid	Mucoadhesive polymers
Dibasic sodium phosphate/monobasic sodium phosphate/calcium chloride/sodium chloride (Caphosol®) supersaturated calcium phosphate/sodium bicarbonate (NeutraSal®)	Electrolyte and pH homeostasis

10.3.3 Medical Devices in Oral Mucositis and Aphthous Stomatitis

Oral mucositis still remains an unmet medical need and even if no effective pharmacological treatment for the prevention and/or treatment are available, several non-pharmacological products/techniques were developed with the specific palliative scope to mitigate the pain induced by the ulcers. In particular the non-pharmacological products/techniques base their activity on mechanical or physical properties and therefore can be enclosed in the medical device classification. In Table 10.3, the mechanisms of actions (MoA) are reported with the scientific rationale claimed.

The coating effect of the devices can be further improved by using polymers with mucoadhesive properties that result in a prolonged effect reducing the removal due to saliva flux and tongue movements. Mucoadhesion can be attributed to polymer properties such as the presence of hydroxylic or carboxylic groups, chain flexibility and good wetting behaviour. Spreadability is a further property important both for mucoadhesion and for easy application on the injured mucosa [4, 37, 40].

10.3.4 Cryotherapy

Cryotherapy has a significant effect on the reduction of oral mucositis for patients receiving chemotherapy; patients are requested to such ice chips for a certain period of time (30–60 min) around the chemotherapy session. This allows decreasing the blood flow with the consequent limitation of the exposure of oral mucosa to the toxic effects of chemotherapy. Patient's compliance is generally good even if some problems are reported in particular in patients who do not accept cold things in the oral cavity and in patients that are treated with oxaliplatin because problems to the exposure, during the chemotherapy, to cold were reported [16].

Table 10.4 Main features and relevant references of products commercially available for oral mucositis treatment

Product	Ingredients
Gelclair® (Helsinn)	Purified water, maltodextrin, propylene glycol, polyvinylpyrrolidone, sodium hyaluronate, potassium sorbate, sodium benzoate, hydroxyethylcellulose, peg-40 hydrogenated castor oil, disodium edetate, benzalkonium chloride, flavouring, saccahrin sodium, glycyrrhetinic acid
Mugard® (access pharmaceuticals)	Purified water, glycerin, benzyl alcohol, sodium saccharin, carbomer homopolymer A, potassium hydroxide, citric acid, polysorbate 60, phosphoric acid
Episil® (Camurus)	Glycerol dioleate, soy phosphatidyl choline (lecithin), ethanol, propylene glycol, polysorbate 80, peppermint oil

10.3.5 Coating Formulations

Coating formulations are characterized by a significant bioadhesion effect, their components are able to bind specifically to the oral mucosa exerting a coating effect that act as a 'fluid plaster' that preserve the ulcerate areas from mechanical, chemical and thermal stimuli that generate pain in patients.

Gelclair®, Mugard and Mucotrol and Episil® are formulations specifically designed to achieve a coating effect of the oral mucosa, although they use different components. Such products are available both in the USA and in EU except for Episil that is available only in the EU.

The main features of these products are given in Table 10.4. In the following section, the results of efficacy studies undertaken on these devices are discussed.

Gelclair® This product helps in the management of painful symptoms of mucositis of the oropharyngeal cavity. Gelclair®, used as a mouthwash, forms a protective film that helps to provide pain relief, and soothing mouth lesions including those caused by medication, disease, radiotherapy and chemotherapy.

Several open label studies ($n=15$) were performed with Gelclair® in patients suffering from oral mucositis mainly from radio and/or chemotherapy; overall in 459 patients, Gelclair® was able to mitigate mucositis symptoms. In particular, the effectiveness of Gelclair® was demonstrated in several clinical trials involving patients affected by inflammatory or ulcerative lesions of the oral cavity caused by chemo and radiotherapy. Gelclair® provided a fast pain relief as demonstrated in the McKenzie study [25] where 115 patients with a confirmed diagnosis of radiation or chemotherapy-induced mucositis benefitted from Gelclair® protective film effect from the first 15 min after application (Fig. 10.4).

In another study [24], 33 patients with oral mucositis caused by radiotherapy for head and neck cancers reported, by the use of a visual numerical scale, a significant reduction (57.8%) of oral pain following Gelclair® treatment. Patients were given Gelclair® for an average period of 2.29 days and, at the end of the treatment, 85% of patients reported overall significant pain improvements from baseline scores

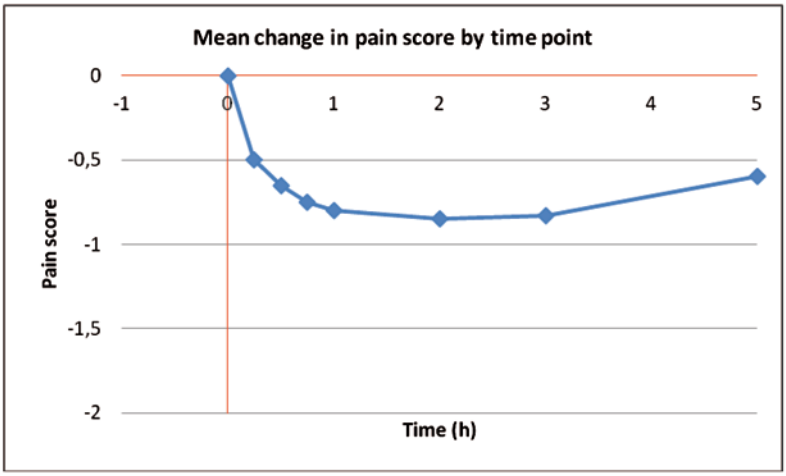

Fig. 10.4 Pain changes after application of Gelclair® in patients with radiation or chemotherapy induced mucositis [25]

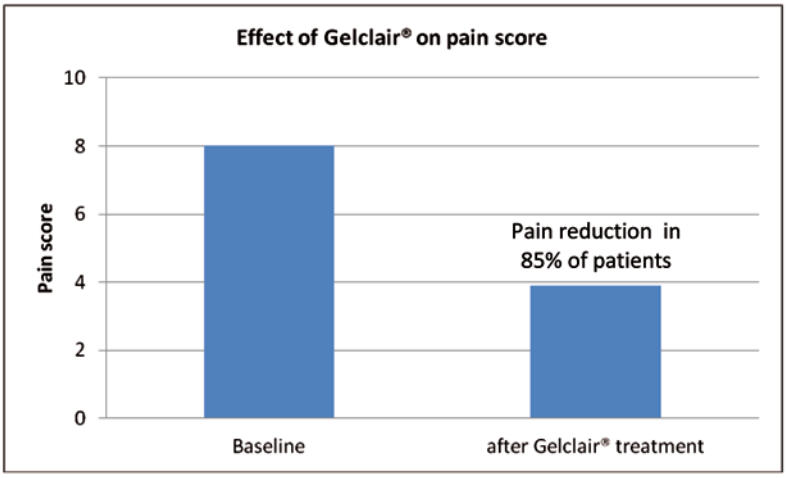

Fig. 10.5 Effects of Gelclair® on pain in patients with oral mucositis [24]

(Fig. 10.5). In another study [8] performed on 53 oncological patients, the median grade of oral mucositis was assessed according to the WHO at different time points. A reduction of 1 grade was recorded (Fig. 10.6) compared to the baseline value, after only three days of Gelclair® treatment. In this study, as in another study in which oral mucositis was developed in 30 patients after chemotherapy [11], the ability to drink and eat was investigated by the use of a numerical scale from 0 to 10 at baseline and after 3 days. It was shown that in both studies patients felt an increase in their quality of life of about 42 %.

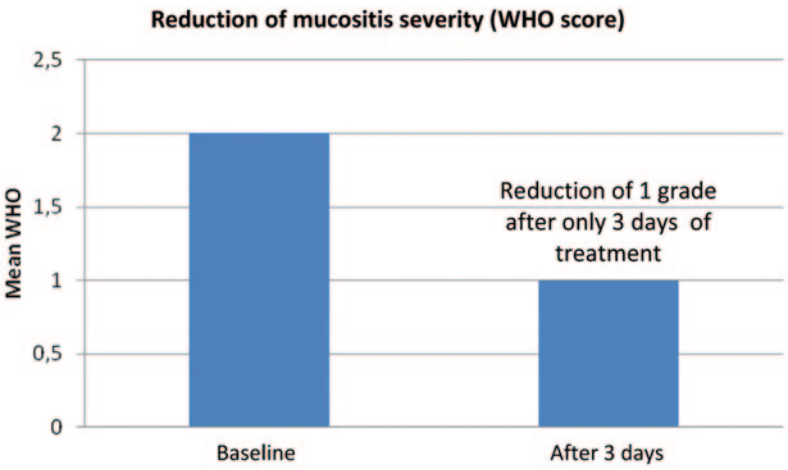

Fig. 10.6 Efficacy of Gelclair® in reduction of mucositis severity (with permission of Helsinn Healthcare S.A.)

In terms of safety, the ingredients of Gelclair® are well known, safe and extensively used in other products. Overall the product is very well tolerated and is non-toxic if accidentally ingested. No interactions are known to exist with other medicinal products. Gelclair® may be used during pregnancy, lactation and in paediatric patients.

MuGard™ This is a mucoadhesive oral wound rinse, is indicated for the management of oral mucositis or stomatitis that may be caused by radiotherapy and/or chemotherapy, and all types of oral wounds (mouth sores and injuries), including aphthous ulcers/canker sores and traumatic ulcers, such as those caused by oral surgery or ill-fitting dentures or braces.

In an open study [9], the efficacy of MuGard was tested in 20 cancer patients.

MuGard delayed the onset of mucositis by up to 2 weeks, compared with what is usually observed in clinical practice, and the median duration of grade 3 oral mucositis was observed at 4 weeks.

In addition there was a reduction in pain identified by limited use of opioids, and a reduction in the severity of clinically assessed and patient-perceived symptoms. In another open label study [30] head and neck cancer patients were treated with MuGard ($n=16$), Caphosol rinsing solution ($n=21$) or standard oral care ($n=48$). This trial was unable to demonstrate that either Mugard™ or Caphosol® improved analgesia score, grade of mucositis or dysphagia compared to an institutional standard mouth care protocol.

Mucotrol The efficacy of Mucotrol, a concentrated oral gel wafer, was tested in a randomized double-blind placebo controlled study ($n=30$) [27]. Eleven patients were evaluated in each arm, and the results showed a significant reduction of study scores (i.e. WHO, RTOG, OSS) in the Mucotrol group with no changes recorded in the placebo group.

As per their characteristics, the use of such devices, in particular Gelclair®, were a source of inspiration for the realization of devices with the intended use niched in the management of minor oral mucosa damages such as aphthae or small damages as a consequence of dental practices.

Electrolyte Solutions Rinsing solutions designed for the management of mucositis (i.e. Caphosol and Neutrasal; see Table 10.3) were developed mainly in order to normalize the electrolytes and pH of the oral cavity.

The presence of inorganic salts in high concentration seemed to improve mucositis' symptoms in patients, even if robust clinical evidences were lacking.

In particular as previously reported, the efficacy of such solutions (i.e. Caphosol) was shown to be comparable to that of Mugard™ but not different from the efficacy obtained with the use of institutional standard mouth care protocols [30].

10.4 Xerostomia

Xerostomia (dry mouth syndrome) is a chronic condition characterized by the decreased or absent production of saliva. The prevalence of xerostomia has risen to 26% in the general population, reaching 82% in the advanced oncology population [10].

Patients suffering from xerostomia generally present a dehydrated oral cavity, with soft tissues that appear particularly dry and atrophic; lips can be fissured and often characterized by the presence of angular cheilosis; the tongue is generally dry with the possible presence of fungal infection; teeth can be seriously damaged.

Among the several causes that can lead to this condition, the most common are autoimmune diseases (Sjögren syndrome), rheumatoid arthritis and iatrogenic conditions, in particular in relation to the use of drugs and cancer therapies [26].

A reduction in the total amount of produced saliva generally causes difficulties in speaking, mastication and consequently bolus production and swallowing. This can be understood when observing all the functions that are related to saliva as illustrated in Fig. 10.7. Patients suffering from xerostomia generally report taste alteration and become particularly sensitive to spicy ingredients. Other symptoms are generally present in patients suffering from xerostomia. In particular, such patients often present teeth caries mainly due to an incorrect hydration, and pH shift to acidic conditions as result of a general modification or the oral micro flora in favour of more cariogenic bacteria [45].

Xerostomia is generally a permanent condition that, on the basis of the severity, can deeply affect the general quality of life of patients. In fact, they are obliged to increase both the frequency and the quantity of liquids during the day in order to mitigate the dry mouth condition, and also sleep conditions are generally negatively influenced by xerostomia.

The first action in xerostomia management consists of the identification—and elimination when possible—of the causes; in particular iatrogenic conditions

Fig. 10.7 The central role of saliva in oral cavity functions

which are sometimes related to specific drugs such as antihistamines, anti-depressives, and drugs for Parkinson's disease. Cancer therapies are other treatments that generally induce xerostomia, and both drugs and radiotherapies are toxic to the salivary glands, with consequent irreversible decrease or total absence of saliva production.

10.4.1 Medical Devices in Xerostomia

Several devices are available in the market for the management of xerostomia; these products base their activity on specific mechanisms of action. These are listed in Table 10.5.

These mechanisms are in line with the regulatory requirements defined for a medical device classification, because they do not act through pharmacological, immunological and/or metabolic properties.

10.4.1.1 Hydration

Patients who lose the capability to produce saliva are obliged to hydrate the oral cavity with external sources of water. Various saliva substitutes are available on the market with the specific aim to have properties which resemble those of natural saliva in terms of chemical and biochemical performances [21]. Even if water is still the main functional component in hydrating the oral cavity, patients report that water per se is less effective than saliva substitutes: this could be mainly related to the low viscosity of water and by the absence, or low levels, of salts and minerals [21].

Table 10.5 Specific mechanisms of actions (MoA) of devices available on the market for the management of Xerostomia

MoA	Rationale
Hydration	Complete substitution of saliva
Lubrication	Integration of the poor quantity of saliva produced
Chemical stimulation	Chemical stimulation of salivary glands
Masticatory stimulation	Mechanical stimulation of salivary glands
Electrical stimulation	Electrical stimulation of salivary glands

Table 10.6 Example of components generally used in saliva substitutes products

Component	Function
Water	Hydration
Carboxymethylcellulose (CMC), hydroxyethylcellulose (HEC), glycerin, hydroxypropylmethylcellulose (HPMC), carmellose salt, glycerate polymer, xanthan gum, polyglycerylmethacrylate (PGM), polyethylene oxide	Viscosity enhancer
Electrolytes including fluoride	Osmolality and electrolytes balance
Enzymes and proteins	Saliva mimetic effects
Aroma	Improve product palatability
Xylitol, sorbitol	Sweetener, anti-cariogenic activity

In Table 10.6 some examples of components—and related function—used in saliva substitutes with hydrating properties are reported. In general these solutions are simple palliative remedies and do not improve significantly the quality of life of the patient, who are obliged to apply the products several times per day with an exacerbation of symptoms during the night. In this respect some intra-oral devices with reservoir of artificial saliva were developed in order to prolong the effects of the solution and consequently decrease the total number of applications during the day [26].

10.4.1.2 Lubrication

These particular classes of product are principally used by patients with a compromised, but not completely blocked, saliva production; therefore the need is only the amelioration of symptoms, or the prolongation of the presence of the low quantity of naturally produced saliva.

The use of high viscosity, non-polar lubrication products exerts a specific effect on the oral mucosa (e.g. Vaseline oil), limiting the dryness process (because it prevents the evaporation of water from the mucosa). Table 10.7 provides examples of components generally used in lubrication gels.

Table 10.7 Example of components generally used in lubrication gels

Component	Function
Water	Hydration
Glycerin 18%, mucin, alginate, Vaseline oil, xanthan gum, linesseed extract, rape oil	Gel constituent
Xylitol, sorbitol	Sweetener

Table 10.8 Most utilized acidic compounds as stimulant of salivation

Component	Function
Citric acid from citrus fruits, ascorbic acid, malic acid from apples and pears	Chemical stimulant of salivation

10.4.1.3 Chemical Stimulation

In order to promote salivary secretion, sucking acidic candies could be of help. Several small studies report the efficacy of this approach on salivary stimulation [21]. Mild organic acidic compounds, such as citric or malic acid, have the direct stimulant effect on the production of saliva by the remaining salivary glands. The most utilized acidic compounds are reported in Table 10.8. The use of lozenges or bio-adherent formulations containing such acidic compounds exerts a positive effect on the mucosa hydration and, moreover, are generally accepted by patients.

10.4.1.4 Masticatory Stimulation

Chewing increases saliva flow both through the stimulation of chemoreceptors that are present in the oral cavity and the stimulation of mechanoreceptors. The use of sugar free chewing gum has been shown to be beneficial and the inclusion of a mild acidic compound in the chewing gum was reported as being more effective compared to the acidic compound alone (lozenges) [1]. Another point in favour of this remedy is that the use of chewing gum is established in daily habits and therefore the acceptance by patients is high.

10.4.1.5 Electrical Stimulation

Xerostomia is the result of a decreased saliva excretion by salivary glands as consequence of a damage of the oral tissues. Salivary glands produce saliva as result of an electrical stimulus coming from the solitary nucleus in the medulla via facial (VII) and glossopharyngeal (IX) nerves [44], therefore relief of xerostomia can be achieved with the external stimulation of such nerves through the electrostimulation of oral mucosa. Such stimulus enhances the production of saliva by salivary glands, with a consequent decrease of oral dryness of patients [2, 20, 44, 47].

The main features and relevant references of the products used as salivary substitutes are given in Table 10.9 which classifies them in terms of their mechanism of action and physical form.

10.5 Innovative Platforms for Medical Devices in Oral Mucosa

The development of innovative platforms, takes advantage of polymeric materials that modify their rheological or mechanical properties in situ, after they have been applied to the oral mucosa. Therefore such vehicles are liquid and easily administered in the form of sprays or mouth-washes which, after the contact with the mucosa surface, transform into gels or films with protective properties. The rheological changes can be induced either by an increase of temperature, from room- to physiological temperature (35–37 °C), or by the presence of the ions that can be found in saliva [13]. The mechanisms involved can be quite different, depending on the polymer. In the case of poloxamers, for example, micelle formation occurs at the critical micellization temperature as a result of PPO block dehydration and, at a definite point, micelles form a gel structure [35]. The gelation of cellulose derivatives with methoxy substitutions (methylcellulose and hydroxypropylmethylcellulose) is based on dehydration and the development of hydrophobic interactions as the temperature increases [41]. Chitosan, has been shown to have thermally sensitive gel-forming properties in mixtures with polyol salts. Some chitosan derivatives, e.g. trimethyl chitosan (TMC) and methylpyrrolidinone chitosan (MPC), have been mixed with glycerophosphate and administered for the treatment of oral mucositis [34, 36]. For other polymers, such as carrageenan, temperature induces a change in conformation whereas cation mediated cross linking is the main reason for ion gelation of alginates and gellan gum [7]. Some examples of in situ gelling polymers are given in Table 10.10.

The healing of a lesion, independently of the etiopatology, progresses through a series of interdependent and overlapping phases in which a variety of cellular and matrix components act together to re-establish the integrity in damaged tissue and the replacement of lost tissue. In this respect an innovative approach is the employment of platelet lysate, a hemocomponent derived from platelets by lysis that is based on a pool of biologically active substances, and in particular of growth factors. Platelet lysate has been shown to exhibit excellent efficacy in repairing different tissues (bones, cartilages) and is used in surgery. It is also recognized that the efficacy of the platelet lysate critically depends on its type (autologous or allogenic) and the way it is made available to the injured tissue. For this reason, recent research conducted in our laboratories is focused on the development of suitable vehicles which are extemporaneously mixed with autologous platelet lysate. Mucoadhesive vehicles (gels and in situ gelling sprays) have been developed to ensure a prolonged contact between damaged mucosa and platelet lysate and to prevent the removal

Table 10.9 Commercial products available for the treatment of xerostomia

Hydration and lubrication		
Product	Ingredients	References
Salivart (Gebauer)	Sodium carboxymethylcellulose, sorbitol, sodium chloride, potassium chloride, calcium chloride and dihydrate, magnesium chloride and hexahydrate, potassium phosphate and dibasic purified water. Propellan: nitrogen	[17]
Oralube (Orion Laboratories Pty Ltd)	Potassium, sodium, magnesium, calcium, chloride, phosphate, fluoride. Methyl hydroxybenzoate, sorbitol	[28, 29, 45]
Biotène Oralbalance Saliva Replacement Gel (GlaxoSmithKline)	Sorbitol, water, glycerin, xylitol, butylene glycol, sodium polyacrylate, polyacrylic acid, hydroxyethylcellulose, sorbi acid, glucose, benzoic acid, lactoperoxidase, lysozyme, lactoferrin, disodium phosphate, glucose oxidase, potassium thiocyanate	[18, 32, 46]
Glandosane® (Kenwood/ Bradley)	Carboxymethylcellulose sodium, sorbitol, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, potassium mono hydrogen, phosphate, sorbic acid, sodium benzoate. Propellant: carbon dioxide	[45]
Electrical stimulation		
GenNarino® (Saliwell)	Active device for non-invasive, short-term applications	[22, 44]
Saliwell Crown® (Saliwell)	Electrostimulating device attached to a regular permanent dental implant	[3]
Masticatory stimulation		
Eclipse (Wm. Wrigley, Jr. Co)	Maltitol, sorbitol, mannitol, aspartame, acesulfame K	
Extra (Wm. Wrigley, Jr. Co)	Sorbitol, mannitol, maltitol, acesulfame K and aspartamel	
Orbit (Wm. Wrigley, Jr. Co)	Sorbitol, mannnitol, xylitol, aspartame, acesulfame K	
Airwaves (Wm. Wrigley, Jr. Co)	Isomalt, sorbitol, mannitol, maltitol syrup (in Honey Lemon only), aspartame, acesulfame K	
Chemical stimulation		
Mouth-Kote Mucopolysaccaharide Sol. (Parnell Pharmaceuticals)	Water, xylitol, sorbitol, yerba santa, citric acid, natural lemon-lime flavour, ascorbic acid, sodium benzoate, sodium saccharin	
Optimoist (Colgate palmo live)	Calcium chloride, citric acid, hydroxyethylcellulose, malic acid, polysorbate 20, sodium benzoate, sodium hydroxide, sodium monofluoroposphate, sodium phasphate monobasic, xylitol	

Table 10.10 Some examples of in situ gelling polymers

Type of gelation	Polymers
Temperature dependent gelation	Poloxamers, xyloglucan, methylcellulose Chitosan and derivatives in combination with beta glycero-phosphate or glyceryl monooleate
Ion dependent gelation	Alginates (gelify in presence of calcium ions), Gellan gum (gelify in presence of cations)

Table 10.11 Composition of innovative vehicle for the delivery of platelet lysate indented for buccal administration

Vehicles	Compositions	Ratio (w/w) of mixing with platelet lysate
PAA (polyacrylic acid) gel	Carbopol 5% (w/w) in saline	1:1
	Saccharin 0.2% (w/w)	
	Flavour 0.2% (w/w)	
	NaOH 4 N to pH 7	
CSG (chitosan glutamate) gel	Chitosan glutamate 6% (w/w)	1:1
	HPMC (K4M) 2% (w/w)	
	Purified water q.s.	
Buccal spray	Poloxamer 407 (Lutrol F127) 14 w/w	1:3
	Sodium Alginate (LVG Pronova) 0.5% w/w	
	Saline solution (0.9% w/v NaCl) q.s.	

effect of biological fluids. Table 10.11 reports the vehicles, their compositions and the w/w ratios of mixtures with platelet lysate used in these studies.

Polyacrylic acid, characterized by its well-known favourable properties such as gel formation, thickening and mucoadhesion; and chitosan, a polysaccharide characterized by mucoadhesive, wound healing, antioxidant, radical scavenger, ROS inhibitor and antinfective properties, have been proposed as buccal gels [38]. Both vehicles were mucoadhesive in the presence of platelet lysate and gave a limited alteration of platelet lysate proliferation activity. This type of vehicle is also the subject of a patent application, licensed to Biomed Device S.r.L. (PCT/IT2008/000744) [5]. The vehicle, based on polyacrylic acid, was then chosen for a preliminary exploratory in vivo study, in the light of the well-established regulatory status of Carbopol® for topical applications [12]. Seven patients, affected by acute or chronic graft versus host disease, with grade II–IV oral mucositis, were enrolled in the study and were treated for 30 days (3 times a day) with a vehicle with a polyacrylic acid composition. Table 10.12 summarizes the preliminary in vivo evaluation which has been reported. Six out of seven patients experienced a benefit. Each showed weight gain and could restart nutrition with solid food, which were considered satisfactory outcomes of the trial.

Table 10.12 Summary of the preliminary in vivo evaluation of PAA vehicle mixed 1:1 w/w with platelet lysate (modified from: [12])

Pts n.	Age	GvHD	Mucositis (grade)	Plt lysate	Weight (% increase)	Response	Use of analgesics	Oral infection
1	13	Acute	IV	allogeneic	0	NR	unchanged	no
2	51	chronic extensive	III	autologous	10	100% R	withdrawal	no
3	34	Chronic extensive	III	allogeneic	7	50% R	reduction	no
4	33	Chronic extensive	III	autologous	2	25 % R	unchanged	no
5	17	Chronic extensive	III	allogeneic	10	100% R	withdrawal	no
6	45	Chronic extensive	II	autologous	3	50% R	reduction	no
7	54	Chronic extensive	III	autologous	6	50% R	reduction	no

a) no response, c) 50% response
b) 25% response; d) 100% response.

During the preliminary in vivo study, even though the polyacrylic acid gel was well tolerated [39], patient compliance was suboptimal due to the difficulty of applying the formulation with a spatula. In order to improve patient compliance and allow easy self-medication, the next step was to develop a buccal spray (pump spraying device) [38]. For this purpose a vehicle characterized by in situ gelation in the physiological environment of the oral cavity was developed. The vehicle was based on a mixture of Poloxamer 407, an amphiphilic thermogelling polymer, known to gel on increasing temperature due to micelle formation and precipitation and sodium alginate, a polysaccharide gelling in presence of calcium ions. After mixing with platelet lysate, the formulation was in the liquid state up to 20 °C and underwent gelification at 37 °C. Thus the vehicle is liquid at room temperature (for an easy sprayability) and gels rapidly on the buccal mucosa at the temperature of about 37 °C. It is also mucoadhesive due to the presence of alginate, which assures a prolonged residence time on the mucosal application site. On the basis of the above results, the thermogelling formulation appeared to be an interesting alternative to the polyacrylic formulation.

At the present time and state of knowledge, the major challenge that remains in the therapeutic use of platelet lysate is to obtain a sterile and safe product using mild preparation conditions. Such products are not necessarily easy to prepare on demand in a hospital pharmacy. Therefore the affordability of a suitable sterile vehicle packaged in suitable monodose container for easy reconstitution of the hemoderivative doses would be extremely helpful to health care providers to permit flexible management of patients. Of course, the in-use stability of the reconstituted product for a certain period of time and under certain conditions must be demonstrated.

Such vehicles in the form of gels, sprays or even films/bandages may be made commercially available as medical devices (typically Class IIb) by specialized laboratories/companies. The commercial availability of these devices would assure the quality and standardization of the final product and facilitate the preparation of study protocols, thus contributing to the feasibility of multicentre clinical trials. In the end, the availability of mucoadhesive/thermogelling vehicles in monodose dispensers to be mixed with platelet lysate resulting in the desired number of doses would result in a simple and economic option for the treatment of difficult-to-treat lesions.

10.6 Concluding Remarks

The opportunities offered by medical devices as a means of supportive care for oromucosal pathologies is an exciting and interesting area of fundamental research and product development. There are still many unmet needs in the treatment of these pathologies. Medical devices provide a potentially useful treatment method and warrant more time and effort to develop improved formulations for the sake of increased patient relief of these, sometimes, debilitating conditions. The development of innovative formulations which take advantage of polymeric materials that modify their rheological or mechanical properties in situ, after they have been applied to the oral mucosa, offers a promising future direction.

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Chapter 11
Pharmaceuticals for Oral Mucosal Drug Delivery: Regulatory Considerations

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The views expressed in this chapter are those of the authors and do not reflect the official policy of the FDA. No official support or endorsement by the FDA is intended or should be inferred.

11.1 Introduction

Oral administration remains the most convenient and preferred method of drug administration. Traditional dosage forms include tablets and capsules, which are intended to be swallowed, with drug absorption occurring primarily in the gastrointestinal tract. Not all drugs, however, are effective when administered this way. Problems associated with the drug (e.g., acid labile and gastric instability) or limitations associated with its metabolism (e.g., first-pass hepatic effects, gut flora metabolism, or low gastrointestinal mucosa permeability) can prove a formidable challenge for some promising therapies. In this regard, the oral cavity has become a very attractive and feasible site for local and systemic drug delivery. Intraoral local drug delivery is a more efficient approach than systemic delivery to treat oral conditions. Likewise, intraoral systemic drug delivery offers several advantages including ease of accessibility, enhanced permeability, avoidance of first-pass metabolism, improved patient acceptability, and increased systemic absorption [1–4]. The terms oral mucosal, oral transmucosal, and intraoral delivery are used synonymously in this chapter to represent drug administration through the oral mucosa.

Despite the many benefits of oral mucosal drug delivery, drug development in this area is not without its challenges. The oral cavity is a complex environment for drug delivery. The term oral mucosa refers generally to the soft-tissue lining of the oral cavity, which includes the lips, cheeks, tongue, gums, hard palate, soft palate, and the floor of the mouth. Drug permeability across the different regions of the cavity varies due to differences in the epithelium thickness and degree of keratinization

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at different sites [1]. Effective drug delivery can be achieved via the buccal, sublingual, palatal, and gingival regions [5]. However, the buccal and sublingual regions are the most commonly used sites for local or systemic drug delivery [4].

Whether the aim is for local or systemic drug delivery, there is a great deal of overlap in the problems associated with formulating products for oral mucosal delivery. Numerous drugs and delivery system platforms have been evaluated for oral mucosal delivery, but only a few drugs have achieved commercial success [6, 7]. Understanding the permeability features of the oral mucosa and the drug's physicochemical properties are critical for selecting an appropriate formulation, as insufficient mucosa permeability could impact the drug's clinical effectiveness. Drug loss from the site of action due to the washing effect of saliva and mechanical stress on the selected dosage form may also be problematic, particularly for adopting conventional tablet dosage forms for intraoral delivery [8]. Over the past few years, technological advances in mucoadhesives, sustained drug release, permeability enhancers, and drug delivery vectors present new opportunities to increase the arsenal of drugs to treat oral and systemic diseases via oral mucosal delivery [6].

Within the USA, the research and development of new intraoral drug products intended for human use, and their marketing, are regulated by the Food and Drug Administration (FDA) under the authority of over 200 laws enacted by the US Congress: most notably, the 1938 Food, Drug, and Cosmetic Act (Title 21 of the US Code) and its numerous amendments, the 2007 Food and Drug Amendments Act (FDAA), and the 2012 FDA and Safety Innovation Act (FDASIA). The regulations define *drug product* as the finished dosage form (e.g., tablet, capsule, etc.) that contains the drug substance, or the pharmacologically active compound, with or without one or more other ingredients. FDA will approve a marketing application for a new drug product only after it determines that the product meets the statutory standards for safety and effectiveness, manufacturing and controls, and labeling. Thus, if the intention of research in oral mucosal drug delivery is to develop a new drug product for human use to treat specific diseases, knowledge of the regulatory considerations and requirements for product development and marketing approval is helpful.

This chapter is intended to provide a general overview of the US regulatory expectations for new intraoral drug products to support the translation of promising drug candidates into consumer products. Since most new medicines in the pipeline for oral mucosal drug delivery are small molecules and not therapeutic proteins, the regulatory concepts presented are tailored to small-molecule drugs and not biologics. However, the general principles are broadly applicable to any new pharmaceutical product.

11.2 Defining a Target Product Profile (TPP)

Because of its complexity, finding new drugs and developing new drug products for consumers are arguably one of the most financially risky endeavors in science, and a major challenge for the pharmaceutical industry. As such, an integrated and

interdisciplinary development plan is warranted to improve one's chance of success. In order to assess the regulatory expectations of a new intraoral drug product, it is helpful to begin by defining the important product design requirements or a TPP. The TPP is a prospective, high-level synopsis of the drug product's key properties, its purpose, and intended clinical use. This synopsis sets the end goals for the development program and should take into account the clinical, chemistry, clinical pharmacology, nonclinical, and biopharmaceutic aspects.

For example, if the purpose of an intraoral drug product is to provide a controlled release alternative to an existing antibiotic drug to treat a local infection in the oral cavity, the clinical and clinical pharmacology requirements for drug approval will be different from a product designed to improve the bioavailability of a treatment for idiopathic Parkinson's disease by avoiding first-pass metabolism effects. Similarly, the chemistry and manufacturing information needed to provide FDA with an acceptable level of product quality assurance to support marketing approval may differ for a novel delivery technology platform utilizing new polymers and complex manufacturing processes compared with one that is modeled after a conventional dosage form such as a tablet. Thus, starting the development program with the end in mind can be a valuable asset for navigating the regulatory hurdles during development.

A list of dosage forms currently approved by FDA for products administered via the oral mucosa, along with some design criteria, are provided in Table 11.1 as a snapshot of delivery platforms with proven success. Of note, a drug's dosage form is based on its appearance and the way it is administered. Consequently, two delivery platforms may be based on fundamentally different release mechanisms, but because they look the same and are intended to be administered the same way, the technology is grouped into the same dosage form class by regulators thereby sharing similar identifying nomenclature. FDA generally defers to the US Pharmacopeia (USP) for drug and dosage form nomenclature standards, which have evolved over the years to harmonize nomenclature across different standards setting bodies and to maintain applicability to evolving science [9]. A granular list of dosage forms may be viewed at FDA's online Data Standards Manual; however, this manual is no longer updated and many of the dosage forms listed are no longer in use [10]. For a historical perspective, however, Table 11.1 specifies the dosage form at initial approval, even though it may not be available for labeling a new drug product as per USP <1151>. For example, Peridex® (chlorhexidine gluconate) mouthwash was approved in 1986 as a mouthwash or oral rinse dosage form. However, mouthwash is not a preferred dosage form according to USP <1151> and similar technology will likely be termed a solution under current naming standards.

Understanding the goals for the selected dosage form (i.e., delivery technology) is only one important component of constructing the overall TPP. Some general concepts to consider when constructing a TPP are as follows:

- Patient population and clinical indications.
- Is there an unmet need in the market for the proposed product?

Table 11.1 Dosage forms used for intraoral delivery and design considerations. (Source: References [9, 10])

Dosage form Route (as applicable)	Definition of dosage form and general regulatory expectations
Tablet	A solid dosage form containing the drug with or without suitable diluents. The tablet may be designed for fast or controlled release
Orally disintegrating	A solid dosage form containing medicinal substances which disintegrates rapidly, usually within a matter of seconds, when placed upon the tongue, releasing the drug which dissolves or disperses in the saliva
Buccal	Buccal tablets are meant to be absorbed through the lining of the mouth without the aid of water and should not be swallowed whole. The tablet dissolves once placed in the upper or lower pouch (buccal pouch) between your gum and the side of your cheek. Patients are generally recommended not to eat, drink, chew, or smoke while the tablet is dissolving. Patients are also often instructed to rinse the mouth and brush their teeth after complete dissolution to remove taste if necessary. (e.g., miconazole buccal tablet)
Sublingual	Sublingual tablets are often fast dissolving, usually a small, flat tablet, and are intended to be inserted beneath the tongue, where the active ingredient is absorbed directly through the oral mucosa. This tablet must not be chewed. (e.g., hyoscyamine sulfate sublingual tablet)
Gums, chewing Buccal	A nondissolving matrix containing the drug and other ingredients that must be chewed but not swallowed to promote release of the drug from the dosage form in the oral cavity. The gum is removed from the mouth and disposed off following use. (e.g., nicotine gum)
Patches Buccal	A nondissolving matrix composed of one or more polymer films or layers containing the drug and/or other excipients. The patch may contain a mucoadhesive polymer layer which bonds to the oral mucosa for controlled release of the drug into the oral mucosa (unidirectional release) or into the oral cavity or release and absorption into the mucosal tissue and oral cavity (bidirectional release; e.g., lidocaine oral patch)
Lozenge	A solid preparation containing one or more medicaments, usually in a flavored, sweetened base which is intended to dissolve or disintegrate slowly in the mouth. A lollipop is a lozenge on a stick. (e.g., fentanyl lozenge)
Gel	A semisolid dosage form that contains a gelling agent to provide stiffness to a solution or a colloidal dispersion. A gel may contain suspended particles. (e.g., benzocaine gel)
Spray	A liquid minutely divided as by a jet of air or steam. (e.g., nitroglycerin spray)
Mouthwash, oral rinse	An aqueous solution which is most often used for its deodorant, refreshing, or antiseptic effect. Generally delivers drug in an uncontrolled manner throughout the mucosa. (e.g., chlorhexidine gluconate mouthwash)

Table 11.1 (continued)

Dosage form Route (as applicable)	Definition of dosage form and general regulatory expectations
Paste	A semisolid dosage form, containing a large proportion (20–50 %) of solids finely dispersed in a fatty vehicle. This dosage form is generally used for local delivery to treat oral conditions. A thin film of the paste is applied to the target area. (e.g., triamcinolone acetonide dental paste)
Films Buccal Sublingual	A fast-dissolving polymer film embedded with drug that melts into the oral cavity quickly and completely releasing the active ingredient for absorption through the oral mucosa. A buccal film will be placed in the buccal cavity, whereas a sublingual film is intended to be placed under the tongue. Though some drug may be swallowed, a large portion is administered via the buccal or sublingual membranes. (e.g., buprenorphine/naloxone sublingual film)

- Drug's physicochemical and biological properties and the desired product characteristics and features—are they compatible?
- Product features that will provide a competitive advantage.
- Ease of product's use, expected patient compliance.
- Studies needed to demonstrate the product's quality, safety, and effectiveness.

A TPP is also a potentially useful communication tool with regulatory authorities. In 2007, FDA proposed a TPP template organized according to the key sections of the product's labeling after discussions between FDA, industry, and academia on ways to improve interactions during the drug-development process. The template is available in the FDA guidance document entitled TPP—A Strategic Development Tool [11]. The key labeling sections are as follows:

- Indications and usage
- Dosage and administration
- Dosage forms and strengths
- Contraindications
- Warnings and precautions
- Adverse reactions
- Drug interactions
- Use in specific populations
- Drug abuse and dependence
- Overdosage
- Description
- Clinical pharmacology
- Nonclinical toxicology
- Clinical studies
- References
- How supplied/storage and handling
- Patient counseling information

From establishing a framework for team members to enabling constructive discussions with regulators, a TPP is a valuable starting point for any new intraoral drug product. With effective use, the TPP can help address issues early on in the drug-development process, thus preventing late-stage drug-development failures and decreasing the overall drug-development timeline. The regulatory requirements for each segment of the development program (e.g., clinical, nonclinical, etc.) may be a standalone chapter if described in detail, and regulatory expectations do vary depending on the clinical targets and type of dosage form. However, there are some common required elements that are generally applicable across all intraoral dosage forms and selected clinical targets. This chapter will touch upon those general requirements.

11.3 Regulatory Pathways for Development

FDA's core administrative and regulatory functions are distributed across four main offices, which are under the direction of the Office of the Commissioner [12]. The key centers and suboffices within these offices are as follows:

- Office of Medical Products and Tobacco
  - Center for Drug Evaluation and Research (CDER)
  - Center for Biologics Evaluation and Research
  - Center for Devices and Radiological Health
  - Center for Tobacco Products
  - Office of Special Medical Programs
  - Office of Combination Products
  - Office of Good Clinical Practice
  - Office of Pediatric Therapeutics
  - Office of Orphan Products Development
- Office of Foods and Veterinary Medicine
  - Center for Food Safety and Applied Nutrition
  - Center for Veterinary Medicine
- Office of Global Regulatory Operations and Policy
  - Office of International Programs
  - Office of Regulatory Affairs
- Office of Operations

The CDER is the primary center within FDA for overseeing the development and marketing of new drug products, generic drugs, and over-the-counter drugs.

To assist in the management of the new drug approval process, along with interpreting and enforcing key provisions of the Food, Drug, and Cosmetic Act, FDA publishes proposed rules or regulations in the Federal Register, which upon finalization, are codified in the Code of Federal Regulations (CFR), specifically Title 21 of the CFR. Table 11.2 lists some key CFR drug regulations to consider during product development.

Table 11.2 Selected drug regulations from title 21 of the code of federal regulations (CFR)

CFR section	Description
21 CFR 50	Protection of human subjects
21 CFR 56	Institutional review board
21 CFR 58	Good laboratory practices for nonclinical laboratory studies
21 CFR 201	Labeling
21 CFR 210	Current good manufacturing practice in manufacturing, processing, packing, or holding of drugs; general
21 CFR 211	Current good manufacturing practice from finished pharmaceuticals
21 CFR 312	Investigational new drug applications
21 CFR 314	Applications for FDA approval to market a new drug
21 CFR 320	Bioavailability and bioequivalence requirements

CFR code of federal regulations

In addition to the CFR, FDA publishes guidance documents describing the FDA's interpretation or policy on various regulatory issues to help industry comply with the regulations. Guidance documents are not legally binding, but do convey the FDA's current thinking on how regulatory requirements can be satisfied. Consequently, FDA's expectations generally do not depart from the available guidance documents without appropriate justification. There are numerous FDA guidances that have been finalized or are in draft form that are accessible to the public on the FDA's website, http://www.fda.gov.

In 1990, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) was established in response to the increasing globalization of drug development. ICH is a joint effort by the USA, European Union, and Japan to achieve greater harmonization in the interpretation and application of technical guidelines and requirements for drug development for better global health. Through this tripartite, several harmonized guidances have been developed and implemented by the FDA. There are four main categories of ICH guidelines addressing both content and formatting requirements: quality, safety, efficacy, and multidisciplinary. ICH guidances provide additional useful resources to navigate the regulatory landscape.

11.3.1 Procedural

Drug development can be organized into four major phases: discovery/design, preclinical, clinical, and marketing. FDA's involvement in the drug-development process begins once early preclinical in vitro and in vivo (animal) studies indicate that the drug or delivery platform has a potential clinical benefit, and human studies are needed to meet the statutory requirements for safe and effective medicines. An investigational new drug application (IND) is the vehicle used to advance product development into the clinical testing phase. It provides notification to FDA of one's intent to conduct clinical studies and requests an exemption from the federal statute

prohibiting interstate commerce of any new drug that is not the subject of an approved marketing application.

The IND regulations are located in 21 CFR 312 and provide guidelines for both content and format. Briefly, an IND should include the following information:

- Form 1571—21 CFR 321.21(a)(1)
- Table of Contents—21 CFR 321.21(a)(2)
- Introductory Statement—21 CFR 321.21(a)(3)
- General Investigational Plan—21 CFR 321.21(a)(1)
- Investigators Brochure—21 CFR 321.21(a)(5)
- Clinical Study Protocol(s)—21 CFR 321.21(a)(6)
- Chemistry, Manufacturing, and Control information (CMC)—21 CFR 321.21(a)(7)
- Pharmacology and Toxicology information—21 CFR 321.21(a)(8)
- Previous Human Experience—21 CFR 321.21(a)(1)
- Any additional information, if applicable, on drug dependence and abuse potential, radioactivity, use in pediatrics, or other pertinent information supporting the safety of the drug—21 CFR 321.21(a)(10)

An IND may be submitted to the FDA at any phase of clinical development, which may vary depending on the drug. Traditionally, clinical studies were categorized into phases based on when the study was completed in development: phase I (dose-tolerance), phase II (dose-response), phase III (placebo or active controlled confirmatory studies), and phase IV (studies after approval). Alternatively, the ICH E8 guidance proposes an alternate classification based on the objective of the studies: human pharmacology, therapeutic exploratory, therapeutic confirmatory, and therapeutic use. The phase classification system, however, is still commonly used and is the language codified in the CFR. Thus, subsequent discussions in this chapter on clinical studies will maintain the phase-oriented classification.

FDA generally provides an IND holder with greater freedom from efficacy considerations during phase I, as long as the investigations do not expose study participants to excessive risks. Patient safety is always a primary consideration regardless of the development phase. In evaluating phase II and III clinical protocols, however, FDA applies greater scrutiny to ensure that the studies are of sufficient scientific quality to yield data that can support marketing approval. If the information submitted in the IND is insufficient to support starting clinical evaluations, FDA has the authority to halt studies by issuing a clinical hold, which could have serious financial ramifications for the IND sponsor. A clinical hold may be issued at the time of the initial IND submission or for a subsequent study protocol submitted to continue clinical development.

To manage regulatory risks and promote transparency, FDA encourages early and open communication between regulators and sponsors regarding the sponsor's development plan. Before initiating clinical studies, a sponsor may request a pre-IND meeting with the appropriate review division to obtain feedback on their development plan or any scientific or regulatory issue that may need to be resolved prior to submitting the IND. Pre-IND meetings can be a useful tool in the overall

product development strategy and may even help with costs and timelines in the following ways:

- Identifying and avoiding unnecessary studies.
- Ensuring that necessary studies are designed to provide useful information.
- Gaining FDA support for a proposed strategy.
- Minimizing the potential for clinical hold.
- Providing an opportunity for creative exchange of ideas.
- Obtaining regulatory insight.
- Minimizing costs.
- Clearly defining endpoints and goals of the development program.
- Allowing early interactions/negotiations with FDA.

An IND holder can request a meeting with FDA staff at any time to discuss development issues during the course of conducting studies under an active IND. However, efficient use of FDA resources generally leads to more efficient drug development. The following meetings at key development milestones are recommended.

- *End of phase 2 meeting*: The objective of the end of phase II meeting should be to determine whether it is safe to begin confirmatory phase III testing. This is also the time to establish an agreement with FDA on the overall plan for phase III (clinical and CMC) and the objectives and design of particular studies. This meeting may help to avoid wasted time and money conducting unnecessary studies because the data requirements to support a future marketing application have been clarified.
- *Pre-NDA*: The objective of the pre-new drug application (NDA) meeting should be to discuss the presentation of clinical, nonclinical, clinical pharmacology, and CMC data (preferably in electronic format) intended to support a future NDA. The meeting helps to uncover any major unresolved problems or issues, to identify studies relied on as adequate and well controlled in establishing the effectiveness of the drug, and to help regulators become acquainted with the data to be submitted for review.

At the conclusion of clinical trials, the NDA is submitted for marketing approval in accordance with the regulations set forth in 21 CFR 314. Although the specific content and format requirements will depend on the drug, intended use, and delivery technology, the NDA should include all relevant data and information collected during research and development to tell the entire drug-development story. Briefly, an NDA generally includes the following:

- Form 356 h—21 CFR 314.50(a)1–5
- Certification Form 3674—Section 402(j) of the Public Health Services Act
- Index—21 CFR 314.50(b)
- Proposed Labeling and Summary Information—21 CFR 314.50(c)
- Technical Sections—21 CFR 314.50(d)
  - Chemistry, Manufacturing, and Controls—21 CFR 314.50(d)(1)
  - Nonclinical Pharmacology and Toxicology—21 CFR 314.50(d)(2)
  - Human Pharmacokinetics and Bioavailability—21 CFR 314.50(d)(3)
  - Microbiology (anti-infective drugs)—21 CFR 314.50(d)(4)

  - Clinical—21 CFR 314.50(d)(5)
  - Statistical Evaluation of Clinical Data—21 CFR 314.50(d)(6)
  - Pediatric Investigations, as applicable—21 CFR 314.50(d)(7)

- Case Report Forms and Tabulations—21 CFR 314.50(f)
- Patent Information—21 CFR 314.50(h)/21 CFR 314.50(i)
- Exclusivity Request—21 CFR 314.50(j)
- Financial Certification/Disclosure Statements—21 CFR 314.50(k)

Upon receipt of the NDA, FDA determines whether the application on its face is complete for review. The amount of required information can be quite substantial and the ICH common technical document (CTD) guidances provide a framework for organizing the information into a modular format to both adhere to statutory requirements and streamline communications with FDA's review staff. Figure 11.1 illustrates the harmonized modular format of an NDA.

Major omissions in the NDA can result in a refusal to file action, which costs time and money. Regulatory risks should be managed accordingly to avoid this pitfall. A refusal to file action is not desirable by regulators, and FDA makes every effort to communicate correctable deficiencies found during the review in a prompt manner to applicants. Maintaining a regulatory response team within one's organization is a useful tactic to ensure timely resolution of the FDA's questions. At the end of the NDA review, FDA takes either an approval or complete response action. The standard regulatory review period is 10 months.

In addition to FDA's regulatory review functions, the FDA is charged with promoting innovation to accelerate patient access to safe and effective products. New indications and drugs employing oral mucosal drug delivery technology may be able to take advantage of a variety of powerful regulatory development tools to help expedite development. There are four main programs aimed at addressing an unmet medical need or treating a serious or life-threatening condition: fast track, breakthrough therapy, accelerated approval, and priority review. Table 11.3 provides an overview of the four expedited program**s.**

It is important to keep in mind that for some disease settings, a drug that is not shown to provide a direct clinical advantage over available therapy may nonetheless provide an advantage that would be of sufficient public health benefit to qualify as meeting an unmet medical need (e.g., ease of administration). Further, FDA permits combining several expedited development and approval methods to help bring medicines to the market as quickly as possible.

Figure 11.2 provides an overview of the general steps involved in taking a potential lead from idea to market, and the key points of interaction with FDA.

11.3.2 New Drugs and Novel Therapeutic Targets

One innovative development path for advancing oral mucosal delivery formulations is to focus on novel, new molecular entities (NME) or new chemical entities (NCE). Developing a new drug product is an arduous, expensive, and complex process,

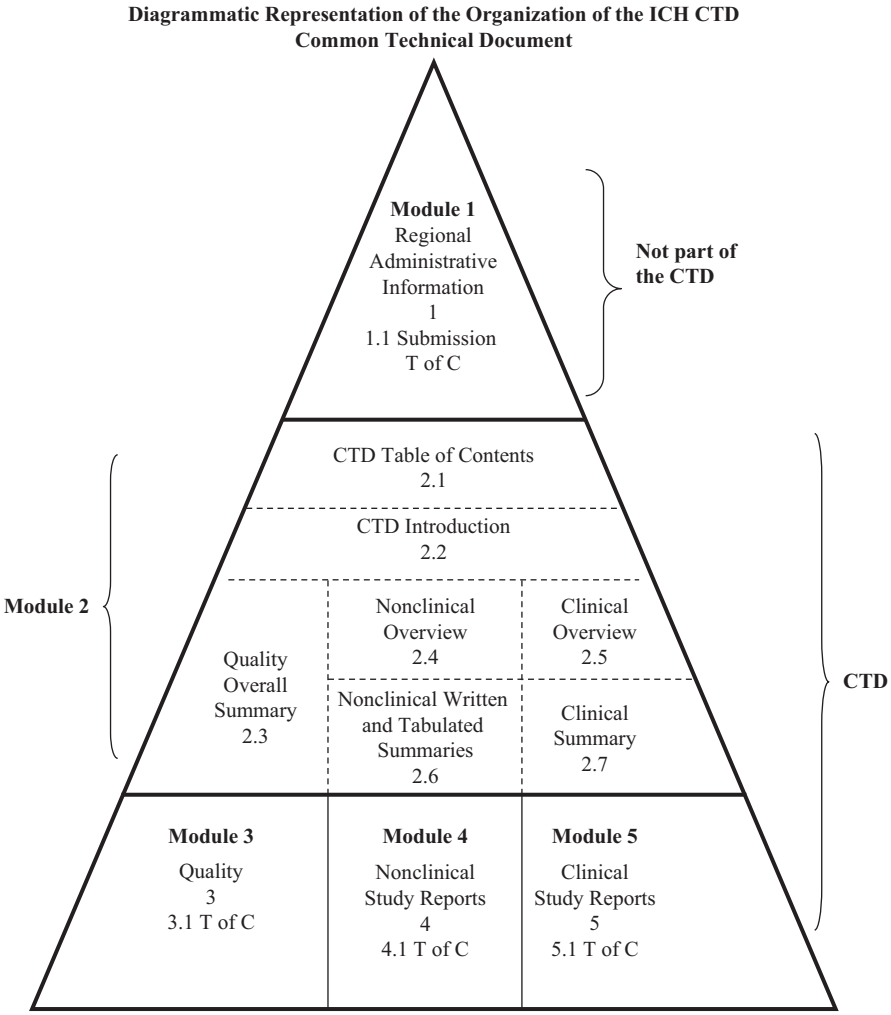

Fig. 11.1 The common technical document format for marketing applications as depicted in the ICH M4—organization of the common technical document for the registration of pharmaceuticals for human use guidance (http://www.ich.org)

which can take as much as 10–15 years of testing, development, and regulatory review under the best circumstances. Yet, it cannot be underscored that the demand for continued development of new treatments is great, given the changing health landscape and socioeconomic burdens of diseases. In 2012, FDA approved 39 novel new medicines, the highest total approved in more than a decade [13]. More than half of these new therapies were first-in-class drugs, meaning the drug targeted a new and unique mechanism of action to treat a particular disorder.

Regulatory incentives to promote research and development of novel therapeutics rely on intellectual property protection and market exclusivity. A patent is a

Table 11.3 Overview of FDA's programs for expedited drug development. (Source: http://www.fda.gov and selected guidances. [11])

	Fast track	Breakthrough therapy	Accelerated approval	Priority review
Authority	Section 506(b) of the FD&C act	Section 506(a) of the FD&C act	21 CFR 314, subpart H Section 506(c) of the FD&C act	Prescription drug user fee act of 1992
Qualification	Serious condition *and* nonclinical *or* clinical data show a potential to address an unmet medical need a qualified infectious disease product	Serious condition *and* preliminary clinical evidence suggest a substantial improvement on a clinically significant endpoint over available therapies	Serious condition *and* meaningful advantage over available therapies *and* demonstrate an effect on an appropriate surrogate endpoint	Serious condition *and* provides a significant improvement in safety or effectiveness a qualified infectious disease product submitted with a priority review voucher
When to request	With IND	With IND	Discuss endpoints with FDA	With NDA
Features	Frequent interactions Rolling review	All of fast track plus intensive guidance Senior management involvement	Approval based on surrogate endpoint	Six-month review clock compared with the standard 10 months

IND investigational new drug application, *FDA* Food and Drug Administration, *NDA* new drug application

property right granted by the US federal government to exclude others from making, using, promoting, or commercializing an invention for a specific time period, usually 20 years. Under the 1980 Bayh–Dole Act, universities and businesses conducting federally funded research can also seek patent protection of applicable early stage discoveries [14]. Utility patents are most applicable to pharmaceuticals and may address concepts such as a novel process, drug, or delivery technology for a particular use. As for exclusivity, an NCE approved by FDA under Section 505(b) of the Food, Drug, and Cosmetic Act is eligible for 5 years of marketing exclusivity. Marketing exclusivity bars any person from submitting an application for a drug product containing the same active moiety as the NCE for 5 years from the approval date of the innovator's NDA, unless the new application contains a certification of patent invalidity or noninfringement, in which case the follow-on applicant may submit after 4 years. New therapeutic targets aimed at orphan diseases are also eligible for 7 years marketing exclusivity for use of the drug to treat the specific orphan disease under the Orphan Drug Act.

Section 505(b) describes two types of NDAs, a 505(b)(1) or 505(b)(2) applications. The two applications must meet the same regulatory standards for approval, but the source of the information relied upon and processing within FDA differs.

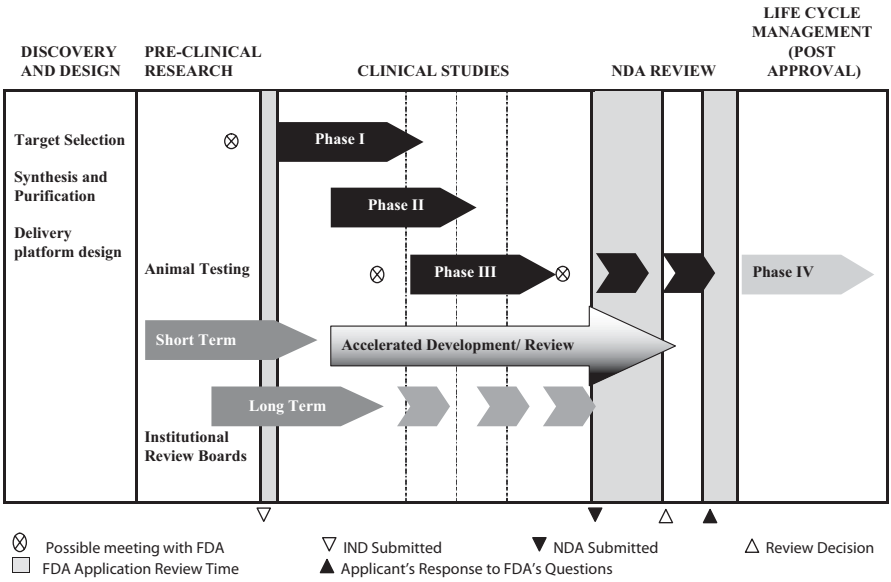

Fig. 11.2 Overview of the FDA drug-development process, adapted from the CDER handbook. (http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM198415.pdf). See also FDA guidance, good review management principles and practices for PDUFA products [11]

Applications submitted under 505(b)(1) contain full reports of safety and effectiveness. The investigations relied upon for approval were conducted by or for the applicant, or the applicant has obtained right of reference for use of the data. A 505(b)(2) NDA also contains full investigations of safety and effectiveness. However, at least some of the information required for approval, usually literature studies for an NCE, comes from studies not conducted by or for the applicant and for which the applicant has not obtained a right of reference. Federal legislation expressly permits FDA to rely on data not developed by the NDA applicant for approval under Section 505(b)(2).

Further, with the enactment of FDASIA, congress renewed the Prescription Drug User Fee Act (i.e., PDUFA V). The PDUFA legislation was first implemented in 1992 and authorizes FDA to collect fees in association with application review. In 2013, the new original NDA fee was $ 1,958,800, which can be a significant allocation of development resources. It may be possible to qualify for a reduced fee or complete waiver if (1) the product has important public health interests, (2) the costs are prohibitive for developing an innovative product or technology, or (3) the company is a small business and is submitting their first marketing application.

PDUFA V also introduced a new review program for NCEs aimed at enhanced transparency and communication between FDA and NDA applicants. The program provides several new changes to the general NDA review process [15]. For example, the regulatory review clock starts after the filing decision date (day 60 after the submission date), which results in an additional 2 months of review time to resolve review issues. Also, the PDUFA V review program includes midcycle and late-cycle

communications with the applicant for improved transparency on the application's status. Managing all development costs and timelines is vital to a successful development program.

11.3.3 Reformulating Existing Products

A potentially more streamlined and equally innovative approach for developing new intraoral drug products is to apply the designated delivery technology to change the route of administration for a currently marketed drug or discover new therapeutic targets for existing products amenable to intraoral administration (i.e., drug repurposing). These approaches may be able to leverage more data generated by other developers under a 505(b)(2) regulatory program than a 505(b)(1) NCE product to meet FDA's expectations for product safety and effectiveness, thereby reducing development timelines.

The 505(b)(2) regulations (codified in 21 CFR 314.54) were promulgated to encourage innovation without creating duplicate work: repeating studies to demonstrate what is already known about a drug simply has no value to both industry and regulators. Much of the same principles under a 505(b)(2) pathway for an approved drug parallels that for generic drugs. That is, a developer can rely on FDA's previous findings of safety and effectiveness for an approved drug. However, federal regulations limit the type of changes that can be approved as a generic drug application under Section 505(j) of the FD&C act. Also, a 505(b)(2) product is considered a new product under the regulations and is subject to PDUFA user fee requirements, regardless of the amount of new information needed for approval.

Both existing nonclinical and clinical data can be leveraged under a 505(b)(2) paradigm for a new intraoral drug product. The type and amount of information that can be leveraged, however, will vary depending on the product's TPP and the quality of the information available for the drug. For example, a drug repurposing approach will likely require new clinical studies to support the new indication, whereas pharmacokinetic (PK) studies may be sufficient for only a change in the route of administration. If, however, clinical studies are necessary for approval of the 505(b)(2) application, the intraoral drug product may be eligible for 3 years of marketing exclusivity. Given that the currently marketed drug may have patent and exclusivity rights to consider, all 505(b)(2) applications need to contain patent certification information.

It is important to understand that the 505(b)(2) pathway does not change the statutory expectation of product safety, efficacy, and quality. It differs only in the source of the information the FDA will use to make its determination of product risks and benefits. So, it is critical to perform a thorough assessment of the information available to support the proposed development plan and to outline what additional information may be needed (i.e., a gap analysis). After outlining the nonclinical, clinical, chemistry, clinical pharmacology, and biopharmaceutics program, a pre-IND meeting with the FDA is recommended to obtain agreement with FDA on the 505(b)(2) development strategy. Reformulating approved drugs has been the primary development strategy for intraoral drug products by the pharmaceutical industry.

11.4 Topic-Specific Considerations and Regulatory Expectations

As noted in previous sections, the specific information required to support marketing approval can be subjected to a case-by-case determination by FDA based on the proposed user population, indication, and complexity of the formulation. However, there are some common regulatory expectations to consider for each technical area. These elements are described in the following subsections.

11.4.1 Clinical Pharmacology and Biopharmaceutics

The in vivo performance of any new drug product should be thoroughly evaluated during drug development by using a systematic set of prospectively planned and appropriately designed studies in order to provide adequate data to support approval. The clinical pharmacology and biopharmaceutics program encompasses those in vivo and in vitro studies evaluating the interplay between the human body and the drug, and how changes in the dosage form or the use of concomitant products (e.g., other drugs, dietary supplements, food, etc.) affect the drug's PK and pharmacodynamic (PD) activity. More distinctly, clinical pharmacology studies focus on defining the drug's mechanism of action and the relationships between dose, drug exposure, and response in the intended patient population, taking into account the various intrinsic and extrinsic factors (e.g., age, race, gender, and drug–drug interactions). The objective of the biopharmaceutics studies, however, is to examine the interrelationship of the physicochemical properties of the drug (i.e., polymorph, solubility, etc.), the dosage form, route of administration and food effect on bioavailability. The biopharmaceutics program, in essence, provides a crucial link between CMC development and clinical efficacy and safety; it is often termed the product quality bioavailability.

11.4.1.1 Clinical Pharmacology

FDA's expectation of what an adequate clinical pharmacology data package should contain has evolved considerably over the past few decades. Regulatory scientists have become more mechanistic in their thinking as technological advances have provided a better understanding of the factors influencing a patient's response to drugs. Consequently, the information in NDA submissions have evolved from an overview of the drug's absorption, metabolism, distribution, and elimination (ADME) profile to more detailed translational analyses that integrates advanced pharmacometrics (i.e., disease modeling), mechanistic safety evaluations, population-based PK, pharmacogenomics, and other kinetic models. Keeping the end goal in mind, however, the regulators key focus with regards to clinical pharmacology evaluations is on drug safety by ensuring proper prescribing information (i.e., the right drug, the right dosing regimen, the right population, supplied in the right form).

The federal regulations define three distinct areas for one to address as part of the clinical pharmacology drug labeling information: mechanism of action, PK, and PD (21 CFR 201.57). Using the labeling requirements as a guide, a general view of the regulatory expectation for a clinical pharmacology development program can be construed. For example, mechanism of action studies should evaluate the drug's action in humans at various levels (e.g., receptor, membrane, tissue, organ, and whole body), where practical. PD studies should evaluate the biochemical or physiologic pharmacologic effects of the drug and active metabolites related to the drug's clinical effect and toxicity. In addition, drug exposure–response relationships (e.g., concentration–response and dose–response) and the time course of PD response (including short-term clinical response) are also important to address. PK studies should evaluate the pertinent ADME parameters. Additionally, bioavailability information should be gathered, with an aim toward defining the following effects and PK parameters, where clinically relevant:

- Plasma PK profile: minimum concentration (C_{min}), maximum concentration (C_{max}), time to maximum concentration (T_{max}), area under the curve (AUC), elimination half-life ($t_{1/2}$), volume of distribution (V_d), time to reach steady state
- Extent of accumulation
- Route(s) of elimination
- Clearance (renal, hepatic, and total)
- Mechanisms of clearance (e.g., specific enzyme systems)
- Drug/drug and drug/food (e.g., dietary supplements and grapefruit juice) PK interactions (including inhibition, induction, and genetic characteristics)
- Linearity/nonlinearity in PK parameters
- Changes in PK over time, and binding (plasma protein and erythrocyte)

It is envisioned that much of the clinical pharmacology information is generated through the classical phase II studies, generally in healthy subjects, that are designed to assess drug tolerance, metabolism, dose response, and other pharmacologic actions of the drug in humans, and, if possible, gain early evidence on effectiveness. Some general study designs include the following:

- Dose-tolerance studies
- Single-dose and multiple-dose PK or PD studies
- Dose proportionality
- Drug–drug interaction studies
- Food effects
- Subpopulation studies (ethnicity, gender, pediatrics, age, etc.)
- Absolute bioavailability
- Relative bioavailability

All clinical studies, regardless of phase, must adhere to high ethical and scientific quality standards to protect the rights and safety of the individuals participating in the studies. FDA, through the ICH, has developed the E6 Good Clinical Practice (GCP) Guidance that should be followed. FDA's bioresearch monitoring program

also conducts on-site inspections of clinical studies supporting IND and NDA applications to verify adherence to GCP guidelines.

The clinical studies are also supplemented with in vitro studies using human material to provide more details on ADME, pharmacologic responses, and interactions. Some general in vitro studies include the following:

- Protein binding
- Liver microsome metabolism
- Transporter studies
- Membrane permeability

For intraoral drug products, the clinical pharmacology program should also consider the following product specific issues in addition to the general clinical pharmacology information.

- Characterization of the site of absorption/permeation for systemic action.
- Evaluating the contribution of buccal or sublingual (i.e., local site within the oral cavity) absorption to the bioavailability.
- Interactions with drugs that influence production of saliva (e.g., anticholinergics).
- Local (e.g., buccal) mucosa irritation.
- Population of subjects for PK studies (i.e., normal subjects vs. patients, pediatric vs. geriatric patients, etc.). The saliva output may be different in young vs. elderly patients.
- The need for PK information in subpopulations such as patients with impaired elimination (renal or hepatic failure), the elderly, children, women, and ethnic subgroups should also be considered.

11.4.1.2 Biopharmaceutics

The goal of the biopharmaceutics program is to identify and evaluate the formulation attributes related to bioavailability that might affect efficacy and/or safety of the proposed drug product (e.g., dosage form/strength proportionality, changes in formulation during clinical studies, etc.) and define appropriate controls (e.g., dissolution) to assure consistent product performance. Bioavailability (BA) data are required for each new drug product submitted in an NDA, or a request to waive the submission requirement for such data, with appropriate justification (21 CFR 320.21). As clinical development proceeds, changes in components, composition, or manufacturing process may necessitate the need for bioequivalence (BE) studies to bridge the clinical data. BA and BE studies both evaluate the rate and extent to which the active ingredient or active moiety is released from the dosage form and absorbed into the body or becomes available at the site of action (i.e., locally acting drugs). In general, each dosage strength is considered a separate drug product for regulatory purposes, and the biopharmaceutics development program should consider the BA data requirement in the context of the entire product design platform.

Several in vivo and in vitro studies are permitted by the regulations to evaluate product quality BA or to establish BE. In descending order of regulatory preference, these are PK, PD, clinical, and in vitro studies. The biopharmaceutics PK and PD studies should be comparative study designs that adopt an equivalence approach to measure the clinical significance of formulation changes. Thus, the study design should include a criterion for comparison, a confidence interval criterion and a BE limit. Unless otherwise indicated in a specific guidance document or agreement with the FDA, the traditional BE limit is a 90% confidence interval between 80.00 and 125.00 for the geometric mean ratios of each specified parameter (e.g., C_{max} and AUC).

For solid dosage forms such as tablets, capsules, wafers, and some films, FDA considers dissolution testing as an acceptable in vitro approach to documenting BA/BE or product sameness under certain circumstances (e.g., for BCS Class 1 drug substance/product, bridging of products with minor formulation and/or process and/or site changes during product development and/or during life cycle management as described in the respective guidances). In particular, an in vitro approach is most justified for a drug that is highly soluble and highly permeable (BCS Class 1) to waive requirements of conducting bioequivalence (BE) studies for new dosage forms (see FDA guidance for waiving studies based on BCS) [11]. Regardless, in vitro dissolution characterization should be done on all product formulations investigated (including early stage prototypes), particularly if corresponding PK is collected for the different formulations. FDA recommends that sponsors consider whether an in vitro/in vivo correlation (IVIVC) model could be developed and used for the proposed drug product as a surrogate for subsequent in vivo BE studies where such studies are necessary to support certain CMC changes. Understanding the relationship between in vitro dissolution and in vivo performance may enable one to develop an IVIVC for regulatory purposes. The most successful IVIVC models have been applied to controlled release dosage forms, but new delivery technologies may be more adept to implementing an IVIVC for a broader range of drug release profiles for regulatory purposes.

Dissolution testing, however, is most commonly used to assess product quality in terms of the formulation's drug release profile, and should be included in the drug product regulatory specification, if relevant to the dosage form. The expectation is that maintaining a consistent in vitro release profiles ensures a low risk of bioinequivalence, or product performance failures, for future manufactured lots. It is simply not feasible to test each manufactured product lot in vivo before distributing the product to consumers. When developing the dissolution method, various dissolution test conditions such as different apparatus (typically USP I or USP II), rotation speeds, and media should be evaluated so that the variability in dissolution rate is minimal and the method results are reproducible. Compendial apparatus (i.e., USP) and methods should be used as a first approach in drug development, and the use of alternative equipment should be considered only when it has been proven that the compendial approaches do not provide meaningful data for a given dosage form. Qualification and validation efforts are expected to demonstrate that the new method is scientifically sound and provides accurate, precise and reproducible data,

assures acceptable drug product quality and allows for some interpretation of the product's in vivo performance. Method validation studies should account for the analytical performance and the ability of the proposed method to detect and reject aberrantly manufactured product. The final method may not necessarily closely imitate the in vivo environment, but should still test the key performance indicators of the formulation from the standpoint of batch-to-batch quality control, and the acceptance criteria should be set keeping in mind not to allow bioinequivalent products in the market.

For rapidly dissolving (>85% dissolved in 15 min at pH 1.2, 4.0, and 6.8) products containing drugs that are highly soluble throughout the physiological range (dose/solubility volume ≤250 mL from pH 1.2 to 6.8), disintegration may be used in lieu of dissolution for quality control testing. A disintegration test is considered most appropriate when a relationship to dissolution has been established or when disintegration is shown to be more discriminating than dissolution. In such cases, dissolution testing may not be necessary. It is expected that development information are included in the NDA to support the robustness of the formulation and manufacturing process with respect to the selection of dissolution versus disintegration testing (see Decision Tree #7(1) in the ICH Q6A Guidance) [11].

11.4.2 Chemistry, Manufacturing, and Controls

Formulations must be designed to produce predictable and consistent systemic drug exposure in the human body. The regulations specify that an NDA must include complete CMC information on both the drug substance and the drug product to support approval. The general regulatory requirements are as follows:

- Drug substance
  - full description of its physical and chemical characteristics
  - stability
  - the name and address of the manufacturing facilities
  - synthetic (or isolation) and purification process
  - process controls used during manufacture and packaging
  - specifications (test methods and acceptance criteria) to ensure the identity, strength, quality, and purity of the drug substance and the bioavailability of the drug products made from the substance
- Drug product
  - list of all components used to manufacture the drug product (regardless of whether they appear in the drug product)
  - the composition of the drug product
  - the specifications for each component of the product
  - the name and address of each manufacturer of the drug product
  - a description of the manufacturing and packaging procedures and in-process controls for the drug product

- the specifications necessary to ensure the identity, strength, quality, purity, potency, and bioavailability of the drug product
- The batch production records for critical bioavailability or bioequivalence study batches and all primary stability batches (note: FDA has historically permitted fewer batch records in submissions and a pre-NDA meeting is a good format to obtain agreement on one's plans for the CMC content in an NDA)
- The proposed or actual master production record, including a description of the equipment, to be used for the manufacture of a commercial lot of the drug product or a comparably detailed description of the production process for a representative batch of the drug product.
- An assessment of the environmental impact from product use

In addition to the CMC information requirements for product quality, manufacturers are also required to comply with the Good Manufacturing Practice (cGMP) regulations codified in 21 CFR 210 and 211. The general cGMP provisions are in 21 CFR 211 and address guidelines for the following: organization and personnel (subpart B), buildings and facilities (subpart C), equipment (subpart D), control of component and drug product containers and closers (subpart E), production and process controls (subpart F), packaging and labeling control (subpart G), holding and distribution (subpart H), laboratory controls (subpart I), records and reports (subpart J), and returned and salvaged drug product (subpart K).

The GMP regulations, however, outline only the minimum requirements for the methods, facilities, and controls used to manufacture the drug product to assure the product's safety, quality, potency, and purity. The lack of specificity in the regulations allows manufacturers the flexibility to decide how best to use modern technologies and innovative approaches to meet product-quality standards, which are reviewed and approved by FDA in the NDA. The downside to generalities is that FDA's expectation of satisfactory cGMP compliance may differ from industry's adoption of the GMP regulations. FDA has taken a number of regulatory actions against drug manufacturers based on the lack of cGMPs to assure product quality. Failure to comply can lead to a not approved action on a pending marketing application. FDA can also issue warning letters, seize products, seek a civil injunction on sales, obtain a consent decree for fines, block imports, or pursue criminal liability for the company or individual employees. Regulatory oversight of pharmaceutical quality continues throughout the product's life cycle.

To address some of the challenges regulators and industry face with pharmaceutical quality, FDA initiated a new initiative in 2002 to modernize the regulation of pharmaceutical manufacturing and product quality: pharmaceutical current good manufacturing practices (cGMPs) for the twenty-first century—a risk-based approach [14, 16]. Through this twenty-first-century initiative, the FDA outlined efforts to reinforce an important principle behind cGMPs; quality cannot be tested into a product, it must be designed and built into a product. The new regulatory review framework for pharmaceutical quality reviews adopts a quality systems view of pharmaceutical quality and applies risk-based and science-based approaches to regulatory decision making. Within this risk-based framework, regulators have

shifted their focus from end-product tests to analyzing the critical in-process controls and quality attributes necessary to provide patients with a reliable product. Manufacturers are strongly encouraged to implement new technologies, such as process analytical technology and incorporate effective tools for knowledge and quality risk management.

The ICH Q8, Q9, and Q10 guidances were developed to assist manufacturers in applying risk based, quality by design (QbD) principles for pharmaceutical quality. Some elements of QbD and risk-based pharmaceutical development to consider are as follows:

- Quality target product profile (QTPP), an extension of the TPP for product quality that identifies the quality characteristics
- Identification of critical quality attributes
- Risk assessment to identify process/product risk
- Design space development
- Control strategy
- Life cycle management

The 2002 cGMP initiative sets the stage for an emerging team approach to quality reviews and facility inspections by FDA, as pharmaceutical quality is one of the FDA's top priorities. Thus, a CMC development program that incorporates some of the science and risk-based principles to development will be better suited to meeting FDA's expectations for higher quality products through scientific innovation.

11.4.3 Nonclinical Pharmacology and Toxicology

In vitro and animal in vivo studies are required to ensure the safety of drugs intended for human use. The nonclinical development program should include a thorough assessment of the drug's toxic effects with respect to target organs, dose dependence, relationship to exposure, and potential reversibility. At the initial IND phase, FDA expects, at a minimum: (1) data on the pharmacological profile of the drug; (2) a determination of the acute toxicity of the drug in at least two species of animals, and (3) short-term toxicity studies ranging from 2 weeks to 3 months, depending on the proposed duration of use in the proposed clinical studies [11, 14, 17]. Animal efficacy studies are generally not needed, but may provide useful data on the drugs mechanism of action and pharmacological effects.

General guidelines for the standard battery of nonclinical tests expected throughout product development are provided in the ICH M3 guidance and include the following:

- Genetic toxicology
- Pharmacology
- Safety pharmacology
- PK/ADME
- General toxicity

- Reproductive and development toxicity
- Carcinogenicity, if relevant to the drug/indication

The nonclinical development program, however, should consider the drug's toxicity relative to the route of administration and intended action. For drugs that act locally, with little to no systemic exposure, the number of studies that are needed to demonstrate drug safety may be reduced.

Intraoral drug products should consider the possibility of drug exposure and toxicities from accidental swallowing as part of the nonclinical development program [11, 14]. If the intraoral drug product is a reformulation of an approved oral dosage form, the previously conducted oral studies to support an oral dosage form may be sufficient to address the regulators' concern regarding swallowing. However, if the intraoral drug product is for a new drug, and there are no previous data to qualify safety from oral dosing, then toxicity studies conducted by the oral route (i.e., gavage, dietary, or drinking water) should be conducted. The optimal design of these studies would include thorough histopathology on the gastrointestinal tract. Regulators are also open to sponsors conducting a 28-day nonclinical oral irritation study of the new intraoral drug product, with an appropriate dosing frequency, as an option to evaluate the potential for excessive local irritation of the oral cavity, in lieu of frequent clinical monitoring.

To ensure the quality of animal safety studies, FDA promulgated the Good Laboratory Practice (GLP) regulations, which are codified in 21 CFR 58. The GLP regulations specify minimum standards for the conduct of safety testing including guidelines on organization and personnel (subpart B), facilities (subpart C) equipment (subpart D), testing and facilities (subpart E), test and control articles (subpart E), protocols (subpart G), records and reports (subpart J), and disqualification of testing facilities (subpart K). Nonclinical studies intended to support marketing applications need to ensure appropriate compliance with the GLP regulations.

Although the GLPs were written to be broadly applicable to a variety of studies, not all GLP provisions apply to all studies. Sponsors may request an exemption from some of the GLP provisions. GLP compliance is expected for core animal safety studies or studies that may provide an important contribution to the drug safety evaluation. PD studies general do not need to be conducted in compliance with GLPs. Even if GLP compliance is not required or done, there should be adequate justification for not adhering to the GLP statutory requirements and a discussion on the potential impact from such conduct on safety pharmacology endpoints. Many nonclinical studies are often contracted out to contract research organizations (CROs). The CRO's GLP compliance status should not be assumed as the regulatory consequences can have significant ramifications on the development program.

11.4.4 Clinical

The results of the clinical trials are arguably the most important factor in the ultimate approval or disapproval of an NDA. A new drug product must provide evidence of safety and effectiveness. The regulatory standard for effectiveness is "substantial

evidence," which is defined in Section 505(d) of the FDC Act as evidence consisting of adequate and well-controlled investigations conducted by qualified individuals. An adequate and well-controlled study has the following characteristics, as defined in 21 CFR 314.126:

- Study protocol with clear objectives and analysis methods.
- A design that permits a valid comparison with an appropriate control for a quantitative evaluation of the drug's effect. Five possible study designs are specified in the regulations.
  - *Placebo concurrent control:* the investigational drug product is compared with an identical drug product containing no drug. Placebo-controlled studies may include additional treatment groups, such as an active treatment control or a dose-comparison control.
  - *Dose-comparison concurrent control*: at least two doses of the drug are compared.
  - *No* treatment *concurrent control*: the test drug is compared with no treatment, but there are objective measurements of effectiveness available and it is known that the placebo effect is negligible.
  - *Active* treatment *concurrent control*: the test drug is compared with known effective therapy.
  - *Historical control*. study data collected for the investigational drug are compared with experience historically derived from the adequately documented natural history of the disease or condition, or from the results of active treatment, in comparable patients or populations. These designs are usually reserved for special circumstances such as studies of diseases with high and predictable mortality.

FDA's general policy is that at least two adequate and well-controlled studies, or pivotal phase III studies, are required to establish efficacy. However, the FDA maintains broad flexibility in defining the clinical data requirements to meet the statutory requirements. For example, existing efficacy data, as in the case of intraoral drug products under a 505(b)(2) development paradigm may allow a sponsor to rely on FDA's previous findings of safety and effectiveness such that only one or no adequate and well controlled pivotal clinical studies are required for approval.

Case Example I: Actiq (fentanyl citrate) oral transmucosal lozenge

Fentanyl citrate is a synthetic opioid analgesic. Before product development, fentanyl had a long history of clinical use in anesthesia and critical care, primarily by intravenous administration. The drug's safety and efficacy profile was well established. Capitalizing on the drug's prior regulatory history, rapid bioavailability and favorable physicochemical properties, the Actiq transmucosal dosage was developed via a 505(b)(2) pathway. The delivery system is relatively simple, a solid matrix formulation of fentanyl citrate on a handle (e.g., lollipop). This unique dosage form, however, is easier for patients to use.

Actiq is approved for the management of breakthrough pain in cancer patients on or tolerant to opioid therapy. After determining the optimal dose for clinical studies, Under the 505(b)(2) paradigm, only one adequate and well-controlled study was required to support approval of this oral transmucosal lozenge.

Case Example II: Zelapar (selegiline hydrochloride) orally disintegrating tablets (ODTs)

Selegiline, which is best known as an irreversible inhibitor of monoamine oxidase (MAO), was developed as an ODT in the treatment of Parkinson's disease.

Zelapar disintegrates within seconds after placement on the tongue and is rapidly absorbed. Detectable levels of selegiline from Zelapar have been measured at 5 min after administration. In addition, the 1.25 mg selegiline ODT produced equivalent exposure to a 10-mg oral conventional dosage form of selegiline. Significant buccal absorption, with the avoidance of first-pass metabolism associated with the conventional oral dosage form, was speculated for enhanced bioavailability and quick onset of action following Zelapar (selegiline hydrochloride).

After determining the optimal dose for clinical studies, the efficacy assessment was based upon two identical, phase 3 randomized, double-blind, placebo-controlled, parallel group multicenter studies, however, the ability to significantly reduce the drug dose through a new ODT formulation with significant buccal absorption provided an important benefit to patients.

Case Example III: Zyprexa Zydis (olanzapine) orally disintegrating tablets

Zyprexa (olanzapine) is a psychotropic agent indicated for the management of the manifestations of psychotic disorders. A conventional Zyprexa immediate release (IR) tablet was approved before the ODT formulation for the same indication. Marketing approval for the conventional IR tablet was supported by the standard battery of clinical studies. Zyprexa Zydis ODT was developed as a line extension based on demonstration of bioequivalence between the Zyprexa Zydis ODT and Zyprexa IR tablets and therefore no clinical trial was conducted for this ODT.

In addition to submitting an IND to initiate clinical studies in the USA, the regulations require approval of the clinical study protocol by an institutional review board (IRB) before the study can be initiated (21 CFR 56.103).

All clinical studies should be designed, conducted, and analyzed according to sound scientific principles. As mentioned previously, clinical trials should also adhere to GCP guidelines, which have their origin in the Declaration of Helsinki: guidelines for clinicians in biomedical research involving human subjects adopted at the 18th General Assembly of the World Medical Association in Helsinki, Finland, in 1964. These principles are outlined in the consolidated ICH E6 guidance and listed below.

1. Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.
2. The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.
3. The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.
4. Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
5. A trial should be conducted in compliance with the protocol that has received prior institutional review board/independent ethics committee approval/favorable opinion.
6. The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.
7. Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
8. Freely given informed consent should be obtained from every subject prior to clinical trial participation.
9. All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation, and verification.
10. The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).
11. Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice. They should be used in accordance with the approved protocol.
12. Systems with procedures that assure the quality of every aspect of the trial should be implemented.

Studies completed exclusively outside of the USA, and not under an active US IND, are still expected to follow GCP guidelines. This standard is intended to protect all subjects in clinical trials and provides regulators with some confidence regarding the quality and integrity of the data. Under 21 CFR 312.120(c)(1), FDA will accept data from a foreign clinical study in support of a marketing application, but if foreign data are the sole basis for the marketing application, the data should be applicable to the US population and US medical practice. In addition, the foreign facilities should be accessible to regulatory inspectors.

11.5 Concluding Remarks

The process of transforming an idea into a consumer product is not always straightforward, and there is a risk of failure at any stage in the process. Investing in research and adopting innovative approaches to intraoral drug delivery may provide an alternate means to maximize commercial success. This approach can take advantage of the known benefits for existing therapies or present a new opportunity to revive a new compound with undesirable PKs via traditional routes. Given the scientific hurdles, which are often unpredictable, a good understanding of the regulatory considerations and requirements for product development goes a long way in helping to take a product from the laboratory to the consumer who needs it.

11.6 List of Guidance Documents

Selected guidance documents by technical area
General
FDA, Target product profile—A strategic development process Tool, (draft) 2007
FDA, Fast track drug-development programs—Designation, development and application review, 2006
FDA, Expedited programs for serious conditions—Drugs and biologics, (draft) 2013
FDA, Formal meetings between the FDA and sponsors or applicants, 2009
ICH M1, eCTD: Electronic common technical document specification, 2003
Clinical pharmacology and biopharmaceutics
FDA, Bioavailability and bioequivalence studies for orally administered drug products, 2003
FDA, Bioanalytical method validation, 2001
FDA, Exposure response relationships—Study design, data analysis, and regulatory applications, 2003
FDA, Dissolution testing of immediate release solid oral dosage forms, 1997
FDA, Waiver of In vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system, 2000
Nonclinical
FDA, Nonclinical safety evaluation of reformulated drug products and products intended for administration by an alternate route, (draft) 2008
FDA, Nonclinical studies for the safety evaluation of pharmaceutical excipients, 2005
FDA, Single dose acute toxicity testing for pharmaceuticals, 1996
ICH S2B, Genotoxicity: A standard battery for genotoxicity testing of pharmaceuticals, 1997
ICH S1A, The need for long-term rodent carcinogenicity studies of pharmaceuticals, 1996
ICH S7A, Safety pharmacology studies for human pharmaceuticals, 2001
Chemistry, manufacturing and controls
FDA, INDs for phase 2 and phase 3 studies chemistry, manufacturing, and controls information, 2003

Selected guidance documents by technical area
ICH Q1A (R2), Stability testing of new drug substances and products, 2003
ICH Q2A, Validation of analytical procedures, 1995
ICH Q3A (R), Impurities in new drug substances, 2008
ICH Q3B (R), Impurities in new drug products, 2006
ICH Q6A, Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances, 1999
ICH Q8 (R2), Pharmaceutical development, 2009
ICH Q9, Quality risk management, 2006
ICH Q10, Pharmaceutical quality system, 2009
ICH Q11, Development and manufacture of drug substances, 2012
Clinical
FDA, Providing clinical evidence of effectiveness for human drugs and biological products, 1998
ICH E6, Good clinical practice: consolidated guideline, 1996
ICH E8, General considerations for clinical trials, 1997
FDA, Acceptance of foreign clinical studies, 2001

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