
9 Basic Biopharmaceutics of Buccal and Sublingual Absorption

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9.1 INTRODUCTION

The quest for alternative modes of drug delivery to the ever-popular oral route of administration has led to exploration of the various mucosae as possible delivery routes. Since the invention of the nitroglycerin sublingual tablet, the oral mucosal route has generated interest as a substitute delivery approach. Recent developments in the design of dosage forms range from tablets and patches to “lollipops” and insertable chips. One of the leading examples of successful buccal delivery includes the oral transmucosal fentanyl citrates (OTFCs). The OTFC in the form of a lollipop, Actiq[®] (Cephalon, Inc., Frazer, PA) has revolutionized the treatment for breakthrough cancer pain relief (BTCR) [1], providing better pain relief as compared to morphine sulfate immediate release (MSIR) tablets. Another novel dosage form is the Periochip[®] (Dexcel-Pharma Ltd., UK), composed of chlorhexidine gluconate in a biodegradable matrix of hydrolyzed gelatin and cross-linked with glutaraldehyde, which is inserted into the intraperiodontal pocket. This has proven to be an excellent treatment for periodontitis, and is often given in conjunction with other dental procedures such as scaling and root planing [2]. Recent investigations have also concentrated on delivery of macromolecules such as peptides using the buccal route. The avoidance of first-pass metabolism and intermediate permeation properties make it attractive for drugs which are sensitive to pH and enzymatic degradation. This chapter aims to provide an overview of the physiology and biopharmaceutics of the oral mucosal route and present an insight into the delivery systems designed for this route.

9.2 PHYSIOLOGY OF THE ORAL MUCOSA

9.2.1 STRUCTURE

The cheeks, lips, hard and soft palates and tongue form the oral cavity. The main difference between the oral mucosa and skin as compared to the gastrointestinal (GI) tract lining lies in the organization of the different epithelia. While the latter has a single layer of cells forming the simple epithelium, the skin and the oral cavity have several layers of cells with various degrees of differentiation.

Within the oral cavity, the masticatory mucosa has a keratinized or cornified epithelium, and covers the stress-enduring regions such as the gingival and the hard palate, providing chemical resistance and mechanical strength. It is divided into four layers: keratinized, granular, prickle-cell, and basal layer (Figure 9.1). The lining mucosa, which provides elasticity, in contrast, is comprised of noncornified surface epithelium covering the rest of the regions including the lips, cheeks, floor of the mouth, and soft palate. It also can be further divided into superficial, intermediate, prickle-cell, and basal layers. The third type of mucosa is the specialized mucosa consisting of both keratinized and nonkeratinized layers, and is restricted to the dorsal surface of the tongue. The intercellular spaces contain water, lipids, and proteins.

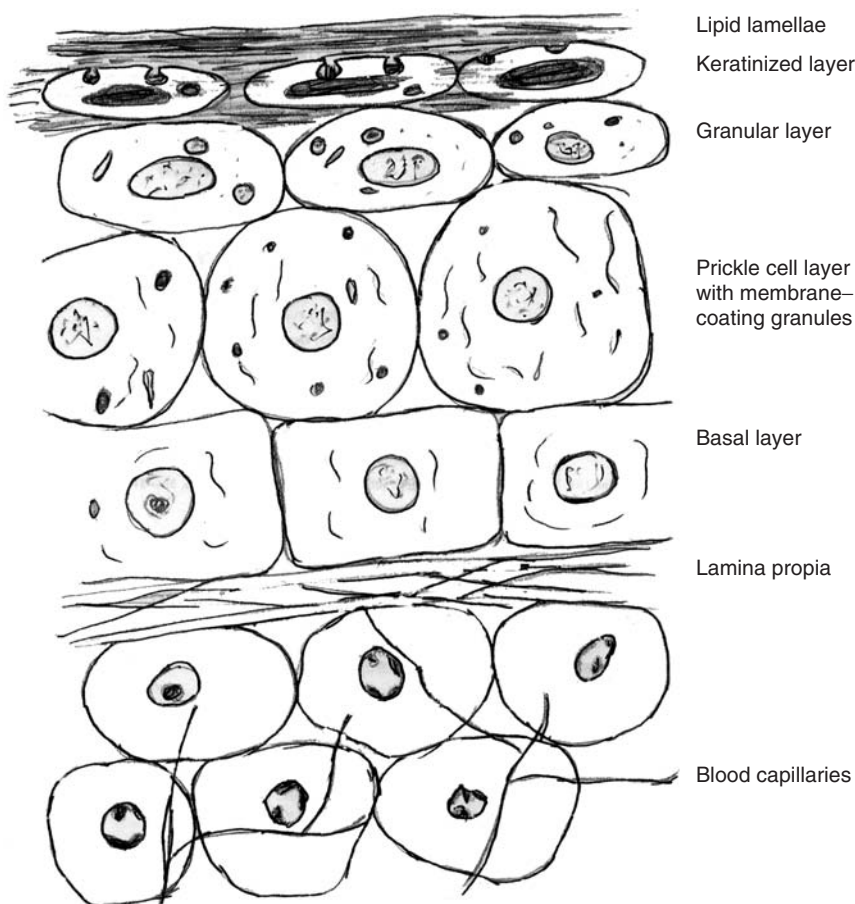


FIGURE 9.1 Structure of the mucosa.

9.2.1.1 Physiological Importance of Mucins and Saliva

The mucosal tissues are further covered with mucus, which is negatively charged, and contains large glycoproteins termed mucins. These are thought to contribute significantly to the viscoelastic nature of saliva, and maintain a pH of 5.8–7.4 [3]. Mucin consists of a protein core, rich in *O*-glycosylated serine and threonine, containing many helix-breaking proline residues. The salivary glands secreting mucus also synthesize saliva, which offers protection to the soft tissues from chemical and mechanical abrasions. The average thickness of the salivary film in the mouth varies between 0.07 and 0.10 mm.

Sustained adhesion of the dosage form (tablet, patch) to the mucosa is an important first step to successful buccal delivery. The mucus plays an important role during this mucoadhesive process by buccal drug delivery systems. The interaction between the mucus and mucoadhesive polymers generally used in most dosage forms can be explained by theories summarized in Table 9.1.

The mean total surface area of the mouth has been calculated to be $214.7 \pm 12.9 \text{ cm}^2$ [4]. The teeth, keratinized epithelium, and nonkeratinized epithelium occupy about 20%, 50%, and 30% of this surface area, respectively.

Drug delivery through the oral mucosa can be achieved via different pathways: sublingual (floor of the mouth), buccal (lining of the cheeks), and gingival (gums). The sublingual

TABLE 9.1
Postulated Mechanisms for Polymer–Mucosal Adhesive Properties

Theory of Adhesion	Mechanism of Adhesion
Adsorption	Secondary chemical bonds such as van der Waals forces, hydrophobic interactions, electrostatic attractions, and hydrogen bonds between mucus and polymer [107]
Diffusion	Entanglements of the polymer chains into mucus network [108]
Electronic	Attractive forces across electrical double layer formed due to electron transfer across polymer and mucus [109]
Wetting	Analyzes the ability of a paste to spread over a biological surface and calculates the interfacial tension between the two [110]. The tension is considered proportional to $X^{1/2}$, where X is the Flory polymer–polymer interaction parameter. Low values of this parameter correspond to structural similarities between polymers and an increased miscibility
Fracture	Relates the force necessary to separate two surfaces to the adhesive bond strength and is often used to calculate fracture strength of adhesive bonds [111]

mucosa is the most permeable followed by the buccal and then the palatal. This is due to the presence of neutral lipids such as ceramides and acylceramides in the keratinized epithelia present on the palatal region, which are impermeable to water. The nonkeratinized epithelia contain water-permeable ceramides and cholesterol sulfate. A comparison of the various mucosae is provided in Table 9.2.

The thickness of the buccal epithelium varies from 10 to about 50 cell layers in different regions because of serrations in connective tissue. In fact, the thickness of buccal mucosa has been observed to be 580 μm , the hard palate 310 μm , the epidermis 120 μm , and the floor of mouth mucosa 190 μm .

9.2.2 TISSUE PERMEABILITY

In comparison to the skin, the buccal mucosa offers higher permeability and faster onset of drug delivery, whereas the key features which help it score over the other mucosal route, the nasal delivery system, include robustness, ease of use, and avoidance of drug metabolism and degradation. The buccal mucosa and the skin have similar structures with multiple cell layers at different degrees of maturation. The buccal mucosa, however, lacks the intercellular lamellar bilayer structure found in the stratum corneum, and hence is more permeable. An additional factor contributing to the enhanced permeability is the rich blood supply in the

TABLE 9.2
Suitability of Various Regions of the Oral Mucosa for Transmucosal Drug Delivery based on Various Tissue Properties

	Permeability	Blood Flow	Residence Time
Buccal	+	++	+
Sublingual	++	--	--
Gingival	--	+	+
Palatal	--	--	++

Source: From de Vries, M.E. et al., *Crit. Rev. Ther. Drug Carrier Syst.*, 8, 271, 1991. With permission.

Note: ++ means very suitable; -- means least suitable.

oral cavity. The lamina propria, an irregular dense connective tissue, supports the oral epithelium. Though the epithelium is avascular, the lamina propria is endowed with the presence of small capillaries. These vessels drain absorbed drugs along with the blood into three major veins—lingual, facial, and retromandibular, which open directly into the internal jugular vein, thus avoiding first-pass metabolism. Numerous studies have been conducted comparing the blood supply of the oral cavity to the skin in animals [5–7]. A thicker epithelium has been associated with a higher blood flow probably due to the greater metabolic demands of such epithelia. Gingiva and anterior and posterior dorsum of tongue have significantly higher blood flows than all other regions; skin has a lower flow than the majority of oral regions; and palate has the lowest of all regions. In fact, the mean blood flow to the buccal mucosa in the rhesus monkey was observed to be 20.3 mL/min/100 g tissue as compared to 9.4 mL/min/100 g in the skin.

9.2.3 BARRIERS TO PERMEATION

The main resistance to drug permeation is caused by the variant patterns of differentiation exhibited by the keratinized and nonkeratinized epithelia. As mucosal cells leave the basal layer, they differentiate and become flattened. Accumulation of lipids and proteins also occurs. This further culminates in a portion of the lipid that concentrates into small organelles called membrane-coating granules (MCGs).

In addition, the cornified cells also synthesize and retain a number of proteins such as profilaggrin and involucrin, which contribute to the formation of a thick cell envelope. The MCGs then migrate further and fuse with the intercellular spaces to release the lipid lamellae. The lamellae then fuse from end to end to form broad lipid sheets in the extracellular matrix, forming the main barrier to permeation in the keratinized regions in the oral cavity. These lamellae were first observed in porcine buccal mucosa [8], and have been recently identified in human buccal mucosa [9]. Though the nonkeratinized epithelia also contain a small portion of these lamellae, the random placement of these lamellae in the noncornified tissue vis-a-vis the organized structure in the cornified tissue makes the former more permeable. Also, the nonkeratinized mucosa does not contain acylceramides, but has small amounts of ceramides, glucosylceramides, and cholesterol sulfate. The lack of organized lipid lamellae and the presence of other lipids instead of acylceramides make the nonkeratinized mucosa more water permeable as compared to the keratinized mucosa.

9.3 ADVANTAGES AND DISADVANTAGES OF BUCCAL AND SUBLINGUAL ROUTES OF ADMINISTRATION

As an alternative method of delivery to the GI route for several classes of pharmaceutical agents, the mucosal route offers protection against low gastric pH, proteases, and first-pass degradation. Proteins and peptides as well as hormones, which are highly pH sensitive, cannot be delivered via the oral route. Avoidance of presystemic metabolism and the near-neutral pH of the oral cavity as compared to the highly acidic pH in the stomach make the buccal route promising for these entities. Peptides can be rapidly metabolized by proteolysis at most sites of administration in the body. Among the mucosal routes, peptide transport through the buccal mucosa was found to be much less sensitive to degrading enzymes as compared to nasal, vaginal, and rectal administration [10]. However, not all peptides escape hydrolysis. Though the buccal mucosa seems to be deficient in proteinases such as pepsin, trypsin, and chymotrypsin present in gastric and intestinal secretions, it is still known to include exo- and endopeptidases, aminopeptidases, carboxypeptidases, deamidases (human, pig, monkey, rat, rabbit, and cultured hamster buccal cells). In addition, saliva also contains esterases and carboxylesterases.

The highly vascularized nature of the buccal mucosa offers the added advantage of fast onset of action. Additionally, patients experiencing difficulty in swallowing, or experiencing nausea, or vomiting can be treated via this route. The noninvasiveness and low level of irritation expected on application also makes the route less intimidating for patients as compared to injections. The lack of need for technical expertise for administration of drugs via this route also makes it a cost-effective alternative to other routes. A relatively high patient compliance due to reduced frequency of dosage administration is another benefit of this route.

The major limitation is that oral mucosa suffers from a relatively small surface area available for drug administration. As mentioned previously, the surface area of the oral mucosa is 200 cm² as compared to the GI tract (350,000 cm²) and skin (20,000 cm²) [11]. Thus, lower amounts of drug are absorbed, rendering achievement of therapeutically efficacious drug concentrations a challenge. Variability in absorption through this route also leads to inconsistent systemic levels. These factors together with peptidases and proteases present in the mucosa lead to systemic drug bioavailabilities of less than 5% of administered dose [12]. Furthermore, the barrier properties of oral mucosa make it a less popular choice as compared to the nasal route. This combined with the small surface area available makes it a satisfactory choice only in the case of highly potent drugs.

Like with any other route of drug delivery, pathological conditions influencing the regular physiology, and functioning of the mucosa will affect the extent and success of the route. Mucosal irritation is another major impediment to the use of buccal route. Such irritation is common because buccal delivery involves applications of drugs, excipients, and enhancers to the mucosal lining for extended periods of time in the form of patches or buccoadhesive tablets.

Other factors which limit mucosal absorption include environmental factors such as the exposure of oral mucosa to salivary flow and the production of shearing forces due to tongue movement and swallowing. Hence in most cases, the actual dose available for buccal absorption is reduced since a high proportion of drug ends up swallowed by the patient.

9.4 PHYSICOCHEMICAL PROPERTIES AND ROUTES OF PERMEATION

Small molecules can be transported across buccal mucosa through two routes: the transcellular (intracellular) route and the paracellular (intercellular) route. Molecules can use either of these two routes or a combination, and the physicochemical properties of the molecule and the mucosal membrane determine the final route of permeation. Hydrophilic molecules can pass through the aqueous pores adjacent to the polar head groups of the lipids in the membrane or through the hydrophilic intercellular cytoplasm. At the same time, lipophilic molecules are likely to use the transcellular route through the lipophilic cell membrane, where the permeation will depend on the partition coefficient. They can also pass through the lipophilic lipid lamellae present between the cells. The intercellular route is a tortuous route and presents a lesser area for the drug to permeate, but has been found to be a predominant route for absorption of many drugs.

Figure 9.2 shows the two routes of permeation that can be used by drugs to pass through the buccal mucosa.

9.5 BIOPHARMACEUTICS OF BUCCAL AND SUBLINGUAL ABSORPTION

9.5.1 PRINCIPLES OF DRUG ABSORPTION

The oral mucosa contains both hydrophilic and hydrophobic components and a combination of both keratinized and nonkeratinized epithelia. Passive diffusion is the most common route

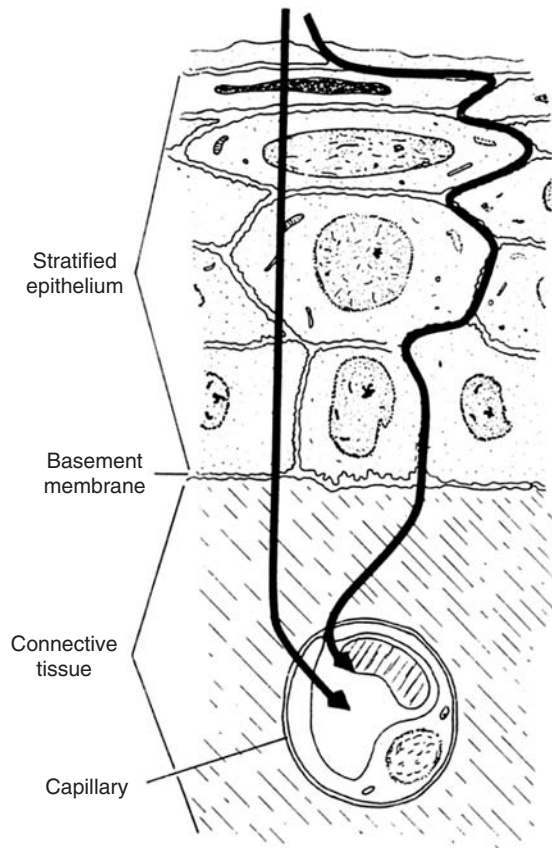


FIGURE 9.2 Routes of transepithelial penetration: transcellular route versus paracellular route. (From Wertz, P.W. and Squier, C.A., *Crit. Rev. Ther. Drug Carrier Syst.*, 8, 237, 1991. With permission.)

of permeation through the oral mucosa, and uses the Fick's first law of diffusion given by the general equation [11]

$$P = \frac{DK_p}{h} \quad (9.1)$$

The amount of drug absorbed A is given by

$$A = PCS t = \frac{DK_p}{h} CS t \quad (9.2)$$

where P is the permeability coefficient, C is the free drug concentration in the delivery medium, D is the diffusion coefficient of the drug in the oral mucosa, K_p is the partition coefficient of the drug between the delivery medium and the oral mucosa, h is the thickness of the oral mucosa, S is the surface area of the delivery or the absorption site on the mucosa, and t is the duration of time the drug stays in contact with the mucosa.

The thickness of the tissue, partition coefficient, and the diffusion coefficient are properties of the mucosa and cannot be altered. Designing appropriate formulations that heed the necessary conditions can vary the surface area for delivery of the drug, time of contact, and the free drug concentration. The partitioning of the drug into the membrane will depend on

its ratio of hydrophilicity and lipophilicity. Studies performed with amines and acids showed that their absorptions were proportional to their partition coefficients, thus also establishing the fact that the transcellular route was the primary route of absorption of these drugs [13]. Similar results were obtained for β -adrenoreceptor-blocking drugs [14]. Since the drug will face different barriers through the paracellular and the transcellular routes, the flux of drug permeation through these routes will differ to some extent. The equation above can be modified to account for this difference.

Hydrophilic compounds will tend to use the paracellular route and permeate through the intercellular spaces, which present a smaller surface area. The flux of drug permeation through this pathway can be described as [15]

$$J_H = \frac{D_H \varepsilon}{h_H} C_D \quad (9.3)$$

where D_H is the diffusion coefficient, h_H is the length of the tortuous path followed in the paracellular route, C_D is the concentration of the drug on the donor side, and ε is the fraction of the surface area of the paracellular route.

A lipophilic drug will preferably use the transcellular route since it will be easier for it to partition into the lipophilic cell membrane. The path length here is shorter than for the paracellular route but the drug has to move through several types of barriers (cell membrane, the cytoplasm, as well as intercellular spaces). Thus the equation for flux through the transcellular route is given as

$$J_L = \frac{(1 - \varepsilon) D_L K_p}{h_L} C_D \quad (9.4)$$

where K_p is the partition coefficient between the lipophilic regions (cell membrane) and the hydrophilic regions (cytoplasm, formulation vehicle, and the intercellular space).

9.5.2 FACTORS AFFECTING DRUG ABSORPTION

Besides the biochemical characteristics of the buccal and sublingual membranes, which are responsible for the barrier function and permeability, various factors of the drug molecule influence the extent of permeation through the membranes.

The lipid solubility, degree of ionization, pK_a of the drug, pH of the drug solution, presence of saliva and the membrane characteristics, molecular weight and size of the drug, various physicochemical properties of the formulation, and the presence or absence of permeation enhancers, all affect the absorption and the permeation of drugs through the oral mucosa.

9.5.2.1 Degree of Ionization, pH, and Lipid Solubility

The permeability of unionizable compounds is a function of their lipid solubilities, determined by their oil–water partition coefficients. Squier et al. [16] demonstrated this dependence of water permeability on the lipid contents of keratinized and nonkeratinized epithelia. The lipids present however contribute to this effect more in the keratinized epithelia (more total lipid content, nonpolar lipids, ceramides) than in the nonkeratinized epithelia where permeability seems to be related to the amount of glycosylceramides present.

The absorption of drug through a membrane depends upon its lipophilicity, which in turn depends on its degree of ionization and partition coefficient. The higher the unionized fraction of a drug, the greater is its lipid solubility. The degree of ionization in turn depends on the pH of the mucosal membrane and the pK_a of the drug. Beckett and Triggs [17] studied

the buccal absorption of basic drugs over a range of concentration, pH, and the use of different drug combinations (alone and mixtures). The resultant pH-absorption curves showed that the percentage of drug absorbed increased as the concentration of drug in the unionized form increased. Also, the shapes of the absorption curves were a function of the pK_a values and the lipid solubility of their unionized form. A study conducted with fentanyl [17], a weak base with a pK_a of 8.2, further demonstrated the relationship between the pH and the absorption across oral mucosa. When the pH of the delivery solution was increased, more of the drug was present in the unionized form, with the drug being 2.45% unionized at pH 6.6, 9.1% unionized at pH 7.2, and 24% unionized at pH 7.7. The fentanyl solutions with a pH range of 6.6 to 7.7 showed a three- to fivefold increase in peak plasma concentration, bioavailability, and permeability coefficients. Similar studies conducted with sublingual administration of opioids such as buprenorphine, methadone, and fentanyl showed increased absorption with increase in pH, where the drug was predominantly present in the unionized form [18]. However, absorption of other opioids such as levorphanol, hydromorphone, oxycodone, and heroin under similar conditions did not improve. These drugs, however, were more hydrophilic as compared to the earlier set of opioids. Thus pH modifiers can be used to adjust the pH of the saliva prior to drug administration to increase the absorption of such drugs through the mucosal membranes.

However, the nature of the buccal and sublingual membrane complicates the above condition since the pH may vary depending on the area of the membrane and also on the layer of the membrane that is considered. The pH of the mucosal surface may be different from that of buccal and sublingual surfaces throughout the length of the permeation pathway [19]. Thus the drug in its unionized form may be well absorbed from the surface of the membrane, but the pH in the deeper layers of the membrane may change the ionization and thus the absorption. Also, the extent of ionization of a drug reflects the partitioning into the membrane, but may not reflect the permeation through the lipid layers of the mucosa. Henry et al. [20] studied the buccal absorption of propranolol followed by repeated rinsing of the mouth with buffer solutions and recovered much of this drug in the rinsing. In addition, the effect of lipophilicity, pH, and pK_a will depend on the transport pathway used by the drug. Studies conducted with busiprone [21] showed that the unionized form of the drug used the more lipophilic pathway, the transcellular route, but an increase in the pH increased the ionization of the drug and subsequently the absorption. It was concluded that this transport of the ionized form of the drug was through the more hydrophilic paracellular pathway. Therefore, at neutral pH the preferred pathway was found to be transcellular, but at acidic pH, the ionized species of the drug also contributed to the absorption across the membrane.

9.5.2.2 Molecular Size and Weight

The permeability of a molecule through the mucosa is also related to its molecular size and weight, especially for hydrophilic substances. Molecules that are smaller in size appear to traverse the mucosa rapidly. The smaller hydrophilic molecules are thought to pass through the membrane pores, and larger molecules pass extracellularly. Increases in molar volume to greater than 80 mL/mol produced a sharp decrease in permeability [22,23].

Due to the advantages offered by the buccal and the sublingual route, delivery of various proteins and peptides through this route has been investigated. It is difficult for the peptide molecules with high molecular weights to make passage through the mucosal membrane. Also, peptides are usually hydrophilic in nature. Thus they would be traversing the membrane by the paracellular route, between cells through the aqueous regions next to the intercellular lipids. In addition, peptides often have charges associated with their molecules, and thus their

absorption would depend on the amount of charge associated with the peptide, pH of the formulation and the membrane, and their isoelectric point (pI).

9.5.2.3 Permeability Coefficient

To compare the permeation of various drugs, a standard equation calculating the permeability coefficient can be used. One form of this equation is [19]

$$P = \frac{\% \text{ permeated} \times V_d}{A \times t \times 100} \quad (9.5)$$

where P is the permeability coefficient (cm/s), A is the surface area for permeation, V_d is the volume of donor compartment, and t is the time. This equation assumes that the concentration gradient of the drug passing through the membrane remains constant with time, as long as the percent of drug absorbed is small. Another approach to determine the permeability coefficient has been described by Dowty et al. [24]. In their studies of the transport of thyrotropin-releasing hormone (TRH) in rabbit buccal mucosa, they incorporated the metabolism of TRH in the rabbit buccal mucosa into the equation, which was given as

$$P = \frac{\left[\frac{d(Q - M)}{dt} \right]}{AC_d} \quad (9.6)$$

where $d(Q - M)/dt$ is the change in quantity of the solute Q minus its metabolites M with time t . A is the exposed area to solute transport and C_d is the concentration of solute in the donor.

9.5.2.4 Formulation Factors

The permeation of drugs across mucosal membranes also depends to an extent on the formulation factors. These will determine the amount and rate of drug released from the formulation, its solubility in saliva, and thus the concentration of drug in the tissues. In addition, the formulation can also influence the time the drug remains in contact with the mucosal membrane. After release from the formulation, the drug dissolves in the surrounding saliva, and then partitions into the membrane, thus the flux of drug permeation through the oral mucosa will depend on the concentration of the drug present in the saliva. This concentration can be manipulated by changing the amount of drug in the formulation, its release rate, and its solubility in the saliva. The first two factors vary in different types of formulations, and the last can be influenced by changing the properties of the saliva that affect the solubility (e.g., pH).

9.5.3 PERMEATION ENHANCEMENT

Enhancers have been used to increase the permeation of drugs through the membrane, and thus increase the subsequent bioavailability. These should be pharmacologically inert and nontoxic, and should have reversible effects on the physicochemical properties of the oral mucosa.

Penetration enhancers have different mechanisms of action depending on their physicochemical properties. Some examples of penetration enhancers and their mechanisms are bile salts (micellization and solubilization of epithelial lipids), fatty acids such as oleic acid (perturbation of intracellular lipids) [25,26], azone (1-dodecylazacycloheptan-2-one) (increasing fluidity of intercellular lipids), and surfactants such as sodium lauryl sulfate (expansion of intracellular spaces). The complete list of enhancers and their mechanism of actions are discussed in detail in Chapter 10.

9.6 IN VITRO AND IN VIVO STUDY METHODS

9.6.1 ANIMAL MODELS FOR STUDIES

The limited available tissue area in the human buccal cavity has encouraged the use of animal models that may mimic human oral mucosal absorption. Rats, hamsters, dogs, rabbits, guinea pigs, and rhesus monkeys have all been used in buccal studies [27–31]. As with any animal model, these all have their advantages and disadvantages. Almost all animals have a completely keratinized epithelium. The hamster cheek pouch offers a large surface area but is not flushed with saliva. The oral mucosa of the monkey, a primate, has been widely used but the high cost of procurement as well as challenging handling are disadvantages when it comes to selecting these animals. Rabbit mucosa is similar to human mucosa since it has regions of nonkeratinized tissue. However, the small surface area and difficulty in accessing the required tissue make it an impractical choice. The animal of choice remains the pig because of comparable permeability to human buccal mucosa and a large surface area enabling reduced variability in the data [32].

The methods used for measuring the amount of drug absorbed have to be designed in such a way as to account for local delivery of the drug to the mucosa as well as systemic delivery through the mucosa into the circulation. A selection of *in vivo* and *in vitro* techniques has been developed and tested over the years.

9.6.2 IN VIVO METHODS

Both human and animal models have been used for *in vivo* testing of oral mucosal drug delivery. Choices of animal models depend on how closely the mucosal membrane reflects the structure and properties of human mucosa. An important *in vivo* technique using human test subjects, the “buccal absorption test” was developed and established by Beckett and Triggs [17]. They adjusted solutions of several basic drugs to various pH values with buffer, and placed the solution in the subject’s mouth. The solution was circulated about 300–400 times by the movement of the cheeks and tongue for a contact time of 5 min. The solution was then expelled, and the subject’s mouth was rinsed with 10 mL distilled water for 10 s. The rinsing was collected, and combined with the earlier expelled solution, and the fraction of the drug remaining in this solution was measured by gas–liquid chromatography. It was observed that the absorption of drug from the oral cavity was dependent on pH. Though this technique is easy to perform, noninvasive, and gives relatively consistent results with little intra- and intersubject variation, limitations for the method do exist [19,33]. It does not provide information concerning the varying permeabilities of different regions in the oral cavity. Also, the continuous flow of saliva affects the pH of the applied solution as well as the overall volume. In addition, the test analyzes the amount of drug that has been transported from the sample into the oral cavity and does not provide information on the actual systemic absorption of the drugs. Some of the drugs could be swallowed or accumulated, and redistributed into the epithelium or biotransformed in the mucosa [34]. Simultaneous measurement of appearance of the drug in the systemic circulation could further validate this test.

“Disk methods” for assessing absorption have also been studied where the drug-loaded disk is kept in contact with certain area of the mucosal membrane to allow for absorption. One such polytef disk was used by Anders et al. [35] for the buccal absorption of protirelin. The disk had an area of ~10 cm² and a central circular depression containing the drug. It was removed after 30 min of contact with the buccal mucosa, and blood samples were taken to determine the amount of drug absorbed from the mucosa.

The disk method provides information about absorption from a specific area of the mucosa. Interference from salivary secretions, difficulties in keeping the disk adhered, and loss of drug permeating due to leakage of the drug from the disk are some disadvantages with this method.

Another method has been the use of perfusion cells [36,37]. These cells have certain specific area and can contain a drug solution that is stirred continuously. The closed cell isolates the solution from the surroundings, thus negating the effects of the environmental factors such as saliva and pH. Solution under test can be passed through the mucosal membrane once or it can be recirculated. The solution in the cells is then analyzed for drug content. However, the surface area for absorption is low, and the tissue has a tendency to become erythematous [38]. This method, like the buccal absorption test, measures the loss of drug from the cell, but not the actual absorption of the drug through the buccal mucosa. These methods have been used to analyze different types of dosage forms (composite films, patches, and bioadhesive tablets) and their mucosal drug absorption and have been used to assess both buccal and sublingual absorptions across the respective mucosa [39].

A glass perfusion cell was developed and used by Yamahara et al. [40] for the measurement of drug absorption through mucosal membranes of anesthetized male beagle dogs. The cell contained a biocompatible bioadhesive polymer O-ring that adhered the cell to the oral mucosal membrane. This type of cell can be used to measure buccal and sublingual absorption as well as perfusion through the surface of the tongue.

9.6.3 *IN VITRO* METHODS

These methods have proven to be important tools in the study of transmucosal absorption, since they can facilitate studies of drug permeation under controlled experimental conditions. Oral mucosal tissue can be surgically removed from the oral cavity of animals. These tissues contain a fair amount of connective tissue, which is separated from the mucosal membrane. This connective tissue, if not removed, may contribute to the permeability barrier. This separation can be carried out with the aid of heat where tissues are separated at 60°C, or chemically by the use of various enzymes or EDTA [41,42]. These tissues are then stored in buffer solution (usually Krebs). This storage step is important in preserving the viability and integrity of the tissue. The tissue is then placed in a side-by-side diffusion cell, where the placement of the tissue is in between the donor and the receptor chambers. The donor contains the drug solution, whereas the receptor usually contains a buffer solution to emulate the body fluids. The chambers can be stirred continuously to ensure even distribution of the drug and are maintained at a desired temperature. The epithelial side of the tissue faces the donor chamber, allowing the drug to pass from the donor chamber through the tissue into the receptor chamber from where samples can be withdrawn at specific time intervals and replaced with fresh receptor solution. A detailed experiment is described in Junginger et al. [43] where transport of fluorescein isothiocyanate (FITC)-labeled dextrans of different molecular weights through porcine buccal mucosa are studied.

Different kinds of diffusion cell apparatus have been used in such *in vitro* experiments. Some of these are small volume diffusion cells as described by Grass and Sweetana [44], Using chambers [45] and Franz diffusion cells [46].

The *in vitro* methods, though relatively simple have various disadvantages: (a) The conditions of tissue separation, preparation, and storage may affect the viability, integrity, and therefore their barrier function. Tests assessing the ATP levels have been used to analyze the viability and integrity of tissue. A method for ATP extraction using perchloric acid and subsequent analysis of ATP in nanomoles per gram of tissue has been described by Dowty et al. [24]. (b) Human oral mucosa is relatively expensive and available in limited amounts. Therefore, animal mucosae which have to be chosen carefully in order to resemble the human mucosa as closely as possible are used. (c) A specific complication occurs in cases of sublingual mucosa. Various ducts from the submandibular and the sublingual salivary glands open into the mucosal surface, and thus a sufficiently large piece of mucosa that is not

perforated by these ducts is difficult to obtain [19]. Also, the presence of enzymes in the tissue indicates that there is a high probability of the drugs being metabolized during transport across the mucosa and therefore appropriate metabolism studies and drug-stabilizing efforts should be undertaken. Studies reported by Dowty et al. [24] measured the extent of metabolism of TRH in rabbit buccal mucosa *in vitro*.

9.6.4 CELL AND TISSUE CULTURE SYSTEMS

The advantages of the *in vitro* approaches described above also apply to buccal cell culture systems. In addition, other aspects such as cell growth and differentiation can be studied in these systems in detail. Also, once the source is established, a continuous supply of cell lines can be obtained, which obviates the need for expensive animal or human tissues that are often difficult to obtain in large quantities.

On the other hand, the established cell line must simulate, as closely as possible, the physical and biochemical properties of the buccal or sublingual tissues *in vivo*. These properties such as the growth, differentiation, biological barrier effectiveness, permeability levels, and metabolic pathways are crucial to the permeation studies.

Of the different types of oral mucosal cell cultures that have been used [47,48], the most commonly used ones are explants of primary cultures. Small pieces of excised buccal or sublingual tissue are placed in a support system and fed with culture medium. The outgrowths obtained from these tissue explants are then transferred and grown in appropriate media. For example, outgrowths of fibroblasts [49] thus obtained have been described. Gibbs and Ponec [50] reconstructed the epithelium of mucosal tissue by placing a tissue biopsy (with the epithelial side upwards) onto a fibroblast-populated collagen gel. The explants obtained were cultured immediately at the air-liquid interface until the epithelium had expanded over the gel (2–3 weeks). These explant cultures may retain many of the *in vivo* tissue characteristics.

Freshly excised buccal or sublingual tissues have also been used to generate dissociated cells. Hedberg et al. [49] used one such culture to measure the expression of alcohol dehydrogenase-3 in cultured cells from human oral mucosal tissue. Human buccal tissue was incubated with 0.17% trypsin in phosphate-buffered saline (PBS) at 4°C for 18 to 24 h to obtain dissociated primary keratinocytes, and subsequently these keratinocytes were seeded onto fibronectin and collagen-coated dishes in serum-free epithelial medium.

Various buccal epithelial cell lines have also been established. The biochemical properties of these cell lines depend greatly upon the growth media and other conditions used during culturing. Hennings et al. [51] showed that the amount of calcium present in the media affects the differentiation of epithelial cells in culture. Different types of cell lines are used for different applications. The TR146 cell line that originated from human neck metastasis of a buccal carcinoma [52] was used as an *in vitro* model of human buccal mucosa to study and compare the enzyme activity with respect to human and porcine buccal epithelium [53]. This cell line has also been used to study and compare the permeability of drugs across cell monolayers, and human buccal tissue to assess the effect of pH and concentration on the permeability [53,54]. The SqCC/Y1 cell line (a squamous epithelial cell line derived from buccal carcinoma) was used to characterize the expression and function of cytochrome P-450s in human buccal epithelium [55].

9.7 DOSAGE FORMS

A wide range of formulations have been developed and tested for buccal and sublingual administration. Various advances have been made over the years, which counteract the

problems faced in delivering drugs through the sublingual and buccal mucosae to the systemic circulation. The primary challenges for these routes of delivery are:

1. The varying structure of the mucosal membrane in different parts of the oral cavity and the reduced permeation due to the barrier presented by the mucosal epithelial layers
2. The constant presence of saliva, which prevents the retention of the formulation in one area of the oral cavity leading to shorter contact time
3. Person to person variability caused by differences in tongue movements, saliva amounts, and saliva content
4. The limited surface area available for absorption
5. Ensuring patient comfort with a dosage form small and flexible enough to fit comfortably in the oral cavity, easy to install and remove, and not causing any local reactions, discomfort, or erythema

Buccal and sublingual deliveries have been used in various clinical applications such as cardiovascular, smoking cessation, sedation, analgesia, antiemesis, diabetes, and hormonal therapy. The specific drugs will be discussed in relation to the dosage form category.

Buccal delivery has also been actively researched for the delivery of peptides, since these molecules are sensitive to the acidic and proteolytic environment of the GI tract and are subjected to first-pass metabolism. The application of this field to peptide delivery will be discussed later in this chapter.

9.7.1 CHEWING GUMS

Gums are now considered pharmaceutical dosage forms, and have been used to deliver drugs for buccal absorption. These formulations consist of a gum base, which primarily consists of resins, elastomers, waxes, and fats. Emulsifiers such as glycerol monostearate and lecithin are added to facilitate and enhance the uptake of saliva by the gum. Resin esters and polyvinyl acetate (PVA) are added to improve texture and decrease sticking of the gum to teeth. Additives such as sweeteners, glycerol (to keep the gum soft and flexible), and flavors can be added as desired [56]. These chewing gums move about in the oral cavity, and the process of chewing mixes it with the saliva where the drug is rapidly released, partitioned, and then absorbed into the mucosal membrane. Thus, the solubility of the drug in saliva is an important factor in increasing the amount of drug released and absorbed. Intersubject variation such as the intensity of chewing, amount of saliva produced, and inconsistent dilution of the drug influence the amount of drug released. Also, the saliva can be swallowed, leading to disappearance of an often unknown amount of drug.

Gum formulations containing caffeine showed rapid release and absorption of the agent with comparable bioavailability to the capsule form [57]. Various gum formulations with vitamin C [56], diphenhydramine [58], methadone [59], and verapamil [60] have been developed and tested.

Recently, sustained release of catechins from chewing gums has been achieved by using a special procedure involving granulation of the active principles with PVA followed by coating of the pellets with acrylic insoluble polymer [61]. One of the most important and successful applications for chewing gum as a dosage form is that for nicotine replacement therapy (NRT) [62]. Nicorette (GlaxoSmithKline, USA), a chewing gum containing nicotine, is available in regular strength (2 mg) and extra strength (4 mg) and has a specially recommended chewing technique to maximize efficacy.

9.7.2 LOZENGES

Lozenges can be used as an alternative dosage form to tablets and capsules when patients are unable to swallow. The use of lozenges has been reported for systemic drug delivery but it is more usual to see this dosage form used to bathe the oral cavity or the throat areas.

While sublingual lozenges may be impractical due to their size, buccal lozenges have been extensively used, and are kept between the cheek and the gums. Though the lozenge usually dissolves in about 30 min, the patient controls the rate of dissolution and absorption because the patient sucks on the lozenge until it dissolves. This process can result in high variability of amounts delivered each time the lozenge is administered. Increases in the amount of sucking and production of saliva may also lead to increased dilution of the drug and often accidental swallowing. In a study conducted by de Blaey and de Haseh [63], there was a noticeable intrasubject variation in residence time (from 2 to 10 min) of unflavored buccal lozenges. They also found that stronger lozenges prolonged the buccal residence time, a factor which can be used as an advantage in local delivery of agents from lozenges.

Despite their drawbacks and an additional requirement of palatability, lozenges have had considerable success in the market. For example, zinc lozenges have been studied and used extensively in the treatment of common colds [64]. A study utilizing NRT was conducted with 2 and 4 mg lozenges. It was found that the lozenges achieved better abstinence from smoking in low- and high-dependent smokers compared to those patients receiving an identical dose in a chewing gum [65]. Transmucosal administration of fentanyl citrate, a medication for breakthrough pain, resulted in a bioavailability substantially greater than oral administration and led to faster achievement of peak plasma concentration [66].

9.7.3 BUCCAL AND SUBLINGUAL TABLETS

These tablets are placed and held between the cheek and gum or the lip and gum (buccal) or under the tongue (sublingual) until they dissolve. Nitroglycerin tablets have been used extensively in the form of buccal and sublingual tablets for the fast onset and quick relief from angina [67,68]. Similarly isosorbide dinitrate is available in the form of sublingual tablets to be placed under the tongue or chewable tablets where the tablet has to be chewed in the mouth for 2 min before swallowing, and the drug is adsorbed through the oral mucosa [69]. Other formulations that have been used are nifedipine (sublingual capsules) [70], sublingual misoprostol for labor induction [71], methyl testosterone (buccal and sublingual tablets), buprenorphine (sublingual and buccal) [34], and selegiline (Zydis selegiline, RP Scherer Corporation, Troy, MI, USA) for monoamine oxidase-B inhibition [72].

9.7.4 MUCOADHESIVE SYSTEMS

One of the primary problems in oral mucosal drug delivery is the retention of the device on the desired area of the membrane for a sufficiently long period of time to allow for absorption of the drug and hence achievement of the desired blood levels. To assist in this, bioadhesive systems have been designed to stay and maintain intimate contact with the mucous membrane that covers the epithelium. These systems are referred to as “mucoadhesive,” and they isolate the delivery of the drug from environmental factors in the cavity and allow the drug to be absorbed only from a specific (buccal or sublingual) region. This results in prolonged contact, and these systems can also be designed to control the release rate of the drug.

Mucoadhesives are generally macromolecular organic polymers made from natural (gelatin, agarose, chitosan, hyaluronic acid) or synthetic polymers (polyvinylpyrrolidone (PVP), polyacrylates, polyvinyl alcohol, cellulose derivatives). They possess hydrophilic groups that can

form hydrogen bonds such as carboxyl, hydroxyl, amide, and amine groups. These mucoadhesives are called “wet” adhesives and need to be in the presence of water in order to hydrate and swell. The amount of water uptake by the system depends on the number of hydrophilic groups in the polymer, and the degree of adhesion in turn depends on the amount of hydration [73]. Upon hydration and swelling, they adhere nonspecifically to the mucosal surfaces. Mucoadhesives can also be used in the dry or partially hydrated forms. Hypotheses have described the mucoadhesion process as initial establishment of contact with the substrate and the subsequent formation of chemical bonds. The attachment to the substrate can be governed by covalent interaction, electrostatic interaction, hydrogen bonding, or hydrophobic interactions. The result is the formation of a tight and intimate contact between the mucosal surface and the polymeric chains of the mucoadhesive, and this “intertangling” between the two surfaces leads to adhesiveness. The mucoadhesion achieved depends on various polymer properties, such as molecular weight, chain length, conformation, and chain flexibility.

Effective mucoadhesion has been used to design different formulations, some of which are discussed below.

9.7.4.1 Films and Patches

Patches are flexible dosage forms that adhere to a specific region of the mucosa and provide either a unidirectional flow or a bidirectional flow of drug, depending on the type of delivery intended (local or systemic). The permeation of the drug into the membrane will depend on the surface area of the patch. Different patches are designed to achieve objectives such as local and systemic drug delivery, varying duration of action and varying rates of release. In general, most patches contain either a “matrix system” in which the drug is dispersed along with excipients or the mucoadhesive, or a “reservoir system.” The mucoadhesive can be dispersed in the drug matrix as described above or as a separate layer. The patches may incorporate a backing layer that protects it from the surrounding oral cavity if strictly transmucosal delivery is required. Otherwise, the backing layer is omitted. The polymer within the mucoadhesive layer swells, and a network is produced through which the drug diffuses into the membrane [74]. Combinations of the above factors have been used to design and develop three kinds of patches: patches with a dissolvable matrix, patches with a nondissolvable backing, and patches with a dissolvable backing. Patches with a dissolvable matrix release the drug into the entire oral cavity, but the presence of a mucoadhesive layer prolongs this release. Patches with a nondissolvable backing provide a unidirectional flow of the drug through the mucosa for a long period of time, whereas patches with a dissolvable backing are short acting as the backing layer dissolves fairly rapidly in the oral cavity [11].

Figure 9.3 shows the two kinds of patch system designs. The patches should be comfortable for the patients to wear for a long period of time, should not hinder or obstruct day-to-day activities, should be easy to attach and remove, and should not cause any local irritation.

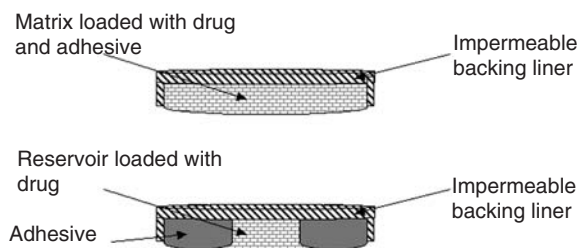


FIGURE 9.3 Alternative matrix and reservoir patch designs. (Modified from Rathbone, M.J., *Oral Mucosal Drug Delivery*, Marcel Dekker, New York, 1996.)

Flexible buccal patches for the controlled delivery of metoprolol, a selective β_1 -adrenergic antagonist, which is widely used to treat essential hypertension, were developed using water-insoluble Eudragit[®] (Röhm GmbH & Co., Darmstadt, Germany) NE40D as the base matrix. Eudragit[®] NE40D is a neutral poly(ethylacrylate methylmethacrylate) copolymer, and is widely used in the development of controlled release delivery systems and film-coating technology [75]. Various hydrophilic polymers, namely Methocel K4M, Methocel K15M, SCMC 400, Cekol 700, Cekol 10000, CP934P, CP971P, and CP974P, were incorporated into the Eudragit[®] patches to modify the drug-release profile and the bioadhesiveness of the buccal patch. Incorporation of the hydrophilic polymers was found to alter both the amount of bioadhesion as well as the drug release [76].

The oral mucosa has also been investigated as a site for immunization, and bilayer films have been developed and administered to rabbits. The films were prepared using different ratios of Noveon and Eudragit[®] S-100 for the mucoadhesive layer and a pharmaceutical wax as the impermeable backing layer. Noveon is a cross-linked mucoadhesive polyacrylate polymer and Eudragit S-100 is an anionic pH-sensitive copolymer of polymethacrylic acid-*co*-methylmethacrylate. The films were pre- or postloaded with 100 g of plasmid DNA expressing β -galactosidase (CMV- β -gal) or β -galactosidase. The films were then applied to the buccal pouch of rabbits and immunological responses were measured. It was found that the weight ratio of Noveon and Eudragit[®] S-100 had a significant effect on adhesion time of the bilayer films. Postloaded films were observed to release 60%–80% of both plasmid DNA and β -galactosidase in 2 h. It was found that this technique of buccal immunization led to comparable antigen-specific IgG titer to that of subcutaneous protein injection [77].

The delivery of buprenorphine, a partial opioid agonist, has been extensively studied using the buccal and sublingual routes since the oral dosage form results in poor bioavailability. In order to increase the retention time on the sublingual membrane, a thin polymeric film consisting of mucoadhesive polymers Carbopol 934P, Carbopol 974P, and the polycarbophil (PCP) Noveon AA-1 was prepared, and polyethylene glycol (PEG) was used as a plasticizer to make the films flexible [78].

A novel buccal delivery system Striant[®] (Columbia Laboratories, Inc., Livingston, NJ) approved by the Food and Drug Administration (FDA) in 2003 is a controlled and sustained release buccal mucoadhesive system, containing 30 mg of testosterone and bioadhesive excipients [79]. The patch contains the bioadhesive polymer PCP, along with other inert ingredients including hydroxypropylcellulose, monohydrated lactose, and cornstarch. After the patch was placed on the gum above the right or left canine, testosterone was slowly released from the matrix. The system was left on for 12 h, then slid out and replaced by another system for the next dosing interval. The testosterone concentrations obtained from the buccal system were found to be within the physiological range for a significantly greater portion of the 24 h treatment period as compared to a marketed testosterone transdermal patch.

9.7.4.2 Tablets

Buccal and sublingual tablets are compressed dosage forms, and like patches can provide either unidirectional flow of drug through the mucosa if they contain a backing layer or bidirectional flow into the oral cavity if no backing is present. The basic formulation is similar to that of patches with a matrix containing the drug, a bioadhesive polymer either in a separate layer or incorporated into the matrix, and the presence or absence of an impermeable backing film.

Recently a study investigated different types of mucoadhesive polymers for buccal tablet formation [80]. The polymers used were Carbopol (CP934 and CP940), PCP, sodium

carboxymethyl cellulose (SCMC) and pectin, all anionic-type polymers, chitosan (cationic type), and hydroxypropyl methylcellulose (HPMC) as a nonionic polymer. These polymers were used alone or in combination to form compressed bioadhesive tablets that were tested for bioadhesion and swelling. Also, residence time *in vitro* was tested using a locally modified USP disintegration apparatus. The polyacrylic acid (PAA) derivatives (CP934, CP940, PCP) showed the highest bioadhesion force and prolonged residence time. While HPMC and pectin demonstrated weaker bioadhesion, SCMC and chitosan showed stronger bioadhesive properties. Among the combinations, a mixture of 5% CP934, 65% HPMC, and 30% spray-dried lactose or 2% PCP, 68% HPMC, and 30% mannitol showed optimal bioadhesion and good residence time.

Bioadhesive tablets can be made by the compression of polymers or can consist of a matrix base or bilayers, with an impermeable backing layer covering the layer with the drug and the mucoadhesion polymer. Examples of these systems are discussed below.

Buccoadhesive-controlled release tablets for delivery of nifedipine were prepared by direct compression of carboxymethyl cellulose (CMC) with carbomer (CP) and compared to those prepared with PVP, PVA, HPMC, and acacia by a modified tensiometry method *in vitro*. It was found that the adhesion force was significantly affected by the mixing ratio of CP:CMC in the tablets. CMC is necessary for controlling the release rate, whereas CP is important in providing bioadhesion. The tablets containing 15% CMC and 35% CP were found to have optimum drug release rate and bioadhesion [81].

Miyazaki et al. [82] designed and evaluated both single and bilayer tablets of pectin and HPMC in the ratio of 1:1 for the sublingual delivery of diltiazem. Bilayer tablets consisted of a backing layer and an adhesive, drug reservoir layer, and were made by covering one side of the single-layer tablet with an inert ethylcellulose layer. The plasma concentration curves for both single-layer and bilayer sublingual tablets showed evidence of a sustained release of diltiazem, with the bilayer tablets with backing layer having a significantly more prolonged effect when compared with single-layer tablets. Bioavailability of diltiazem was 2.5 times that achieved by oral administration for single-layer tablets and 1.8 times for the bilayered tablets.

Biphasic buccal adhesive tablets have also been used for smoking cessation therapy with nicotine [83].

In order to improve the mucosal absorption of poorly absorbed drugs such as peptides and proteins, newer delivery systems with higher mucoadhesive and permeation-enhancing polymers have been developed. While the first generation of mucoadhesive polymers provided adhesion to the mucus gel layer via secondary bonds, the new generation of mucoadhesive polymers is able to form covalent bonds with the mucous layer. The immobilization of thiol groups on mucoadhesive polymers results in thiolated polymers or thiomers that can form disulfide bonds with cysteine-rich subdomains of mucus glycoproteins [84,85].

Langoth et al. [86] studied the properties of matrix-based tablets containing the novel pentapeptide leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) that has been shown to have pain-modulating properties. The matrix-based tablets were made with the thiolated polymer PCP. The covalent attachment of cysteine to the anionic polymer PCP leads to an improvement of the stability of matrix tablets, enhances the mucoadhesive properties, and increases the inhibitory potency of PCP towards buccal enzymes. All these factors lead to stability of the peptide and a controlled drug release for the peptide was obtained for more than 24 h. Also, the tablets based on thiolated PCP remained attached on freshly excised porcine mucosa 1.8 times longer than the corresponding unmodified polymer.

Solubilization of poorly water-soluble drugs by complexation with cyclodextrins and then delivery via the buccal or sublingual mucosa has been studied as an additional strategy for increasing drug absorption. Cyclodextrins are able to form inclusion complexes with drugs,

and can increase the aqueous solubility, dissolution rate, and bioavailability. Jug and Becirevic-Lacan [87] studied the drug carrier system of a molecular complex of piroxicam with hydroxypropyl β -cyclodextrin incorporated in a hydrophilic matrix. The buccal tablets were prepared by a direct compression of HPMC and Carbopol 940 (C940). The *in vitro* release results demonstrated that complexed matrix tablets displayed faster piroxicam release compared to those containing free drug. The combination of HPMC and C940 was shown to demonstrate good bioadhesion properties.

Buprenorphine films prepared with the polymers Carbopol 934P, Carbopol 974P, and PCP Noveon AA-1 were compared to similar mucoadhesive sublingual tablets by Das and Das [78]. The tablets were prepared with or without excipients, and the mucoadhesive properties were studied. It was found that the mucoadhesive tablet formulations produced overall superior results compared to the mucoadhesive film formulations, and optimum results were reported in the case of high lactose, low mucoadhesive polymer, Carbopol 974P- and PEG 3350-containing tablet formulations. These formulations provide a sustained release profile of the drug without producing any sudden “burst release” effects. Also, the tablets were capable of releasing their entire drug content within 2 h, which is optimal for sublingual administration.

9.7.4.3 Hydrogels

Hydrogels are three-dimensional, hydrophilic, polymeric networks that can take up large amounts of water or other biological fluids. The networks consist of homopolymers or copolymers having physical or chemical cross-links that make them insoluble, which are responsible for the integrity of the network.

Depending on their chemical side groups, hydrogels can be neutral or ionic. For a hydrogel to possess mucoadhesive properties, the polymer chains have to be mobile to facilitate the interpenetration into the mucous layer and formation of bonds leading to mucoadhesion. Absorption of water by the hydrogel results in lowering of the glass transition temperature (T_g), and the gel becomes more rubbery. This leads to increased mobility of the polymer chains and establishment of mucoadhesion. The swelling of a hydrogel depends on the properties of the hydrogel itself or properties of the changing external environment. The cross-linking ratio (the ratio of the moles of cross-linking agent to the moles of polymer-repeating units) is one of the primary factors affecting the swelling [88]. The higher the amount of cross-linking agent, the greater is the ratio, thus leading to a tighter structure which leads to less mobility of the polymer and lesser swelling. Also, gels containing more hydrophilic groups will swell more as compared with those containing more hydrophobic groups.

Swelling of physiologically responsive hydrogels is affected by various external factors such as pH, ionic strength, temperature, and electromagnetic radiation [89].

The drug can be either present in a matrix core anchored by a hydrogel to the mucosa or it can be dispersed into the mucoadhesive matrix. In the second case, swelling will play a primary role in the release of the drug from the system.

de Vries et al. [90] determined the adhesiveness of the copolymer hydrogels made of acrylic acid (polar) and butyl acrylate (apolar) in different molar ratios to porcine oral mucosa. Azo-*bis*-isobutyronitrile was used as the polymerization initiator, and ethylene glycol dimethacrylate was used as the cross-linker in varying concentrations. The glass transition temperatures and the water contact angles were measured to indicate the mobility of the polymer chain and the extent of surface polarity of the hydrogel, respectively. The peel and shear detachment forces from the mucosa were determined for the copolymers, which are directly related to the extent of adhesiveness. It was found that the contact angle maximized at

50% butyl acrylate content, whereas the glass transition temperatures decreased as the concentration of butyl acrylate was changed from 0% to 100%. The data indicated that not only a low T_g , but also an optimal number of polar groups, are necessary for optimal adhesion to the mucosal surface.

A study compared the buccal mucoadhesive properties for different polymeric films that differed in their cross-linking status [91]. Synthetic (Carbopol 971P, PCP), semisynthetic (SCMS), and natural carrageenan (λ -type) were analyzed for their mucoadhesive properties using a TA-XT2i texture analyzer.

The texture analyzer gave detachment profiles of these polymers from bovine sublingual mucosa after mucoadhesion under a force of 0.5 N for periods of 0.5, 2, 15, and 30 min, with the polymeric film of PVP K-90 used as a control. Rheological examinations, torque sweep, frequency sweep, and oscillatory examinations were also conducted. In addition, swelling properties were determined with weight measurement before and after wetting with saliva. After a contact time of 2 min, the strength of mucoadhesion was established as $CMC > PCP > Carbopol\ 971P > Carageenan$. But after a contact time of 15 min, the order was reversed to $Carbopol\ 971P > PCP > Carageenan > CMC$. The swelling of the polymers at 2 and 15 min showed the same reversal of order. Thus as compared to CMC, the other three polymers were found to have good mucoadhesive and swelling properties. The study also emphasized the importance of the composition of the chains, the charge density, and the molecular weight to form a network that is capable of forming relatively strong links with the mucous membrane.

Copolymers of acrylic acid and poly(ethylene glycol) monomethylether monomethacrylate (PEGMM) were used to design a buccal delivery system for the systemic delivery of the antiviral agent, acyclovir [92]. The system consisted of the copolymer, an adhesive, and an impermeable backing layer to allow strictly unidirectional flow of the drug. The drug was loaded by equilibrium swelling of the copolymeric films in isotonic buffer (pH 6.8) solutions at 37°C for 24 h. Permeation studies through porcine buccal mucosa were carried out using side-by-side flow through diffusion cells (Crown Glass Co., NJ). It was found that buccal permeation of acyclovir from the mucoadhesive delivery system was controlled for up to 20 h with a time lag of 10.4 h and a steady-state flux of 144.2 $\mu\text{g}/\text{cm}^2/\text{h}$. With the incorporation of sodium glycocholate (NaGC) as a penetration enhancer, the lag time was decreased to 5.6 h, and the steady-state flux increased to 758.7 $\mu\text{g}/\text{cm}^2/\text{h}$.

Hydrogels have also been used to deliver drugs *in vivo* through the oral mucosa. One such example is the preparation of a hydrogel containing 17- β -estradiol, which is administered for osteoporosis, but has very poor oral bioavailability [93]. The hydrogels were prepared by mixing an ethanolic solution containing the drug and an absorption enhancer with an aqueous solution of carboxyvinyl polymer and triethanolamine to produce an ointment. The buccal administration of the hydrogel formulation containing the estradiol in 40% (w/w) ethanol and using 2% (w/w) LAU (glyceryl monolaurate) as the absorption enhancer allowed the maintenance of the plasma level at above 300 ng/(mL cm^2) for 7 h.

9.7.5 OTHER DOSAGE FORMS

9.7.5.1 Sprays

These can be sprayed orally onto the buccal or the sublingual membrane to achieve a local or a systemic effect. One such spray called insulin buccal spray (IBS) was developed with soybean lecithin and propanediol [94]. Soybean lecithin has high affinity for biomembranes but does not enhance the transport of drugs due to low solubility. Propanediol can improve the solubility of soybean lecithin, and act as an enhancer. IBS was administered to diabetic

rabbits, and the hypoglycemic effect of this formulation was investigated. The results show that when the diabetic rabbits were administered with IBS in dosages of 0.5, 1.5, and 4.5 U/kg, the blood glucose level decreased significantly compared with that of the control group, and the hypoglycemic effect lasted over 5 h. To investigate the transport route for insulin through the buccal mucosa, penetration of FITC-labeled insulin was studied by scanning the distribution of the fluorescent probe in the epithelium using confocal laser scanning microscopy. The results revealed that FITC-insulin can pass through the buccal mucosa promoted by the enhancer and the passage of insulin across the epithelium involved both intracellular and paracellular routes.

9.7.5.2 Carrier-Associated Suspensions

Another novel approach to buccal administration of insulin involves using insulin associated with a carrier, namely erythrocyte ghosts (EG) [95]. The insulin was administered either free or attached to carrier systems (erythrocyte ghosts-insulin, EG-INS) to streptozocin diabetic rats by instilling the dose in the mouth cavity using a syringe. To prevent swallowing of the dose, the rats were anesthetized, and blood samples were collected from the tail over 5 h. The magnitude of blood glucose level decline was found to be at its maximum of 39.53 mg/dL (at 2 h) for free insulin and 26.23 mg/dL (at 4 h) for EG-INS insulin, showing that the carrier-associated system was significantly effective at decreasing the blood glucose levels.

9.7.5.3 Liposomes

Liposomes have been used in the local delivery of drugs to the oral mucosa. Farshi et al. [96] studied the biodistribution of dexamethasone sodium phosphate (DSP) encapsulated in multilamellar vesicle (MLV) liposomes labeled with ^{99m}Tc in ulcerated and intact oral mucosae of rats. The liposomes were found to localize the drug in the ulcerated area and increase local drug concentration while decreasing systemic concentration.

Yang et al. [97] investigated the effect of deformable lipid vesicles as compared to conventional vesicles for delivering insulin to the buccal mucosa. The deformable lipid vesicles also called “transfersomes” contain at least one inner aqueous compartment, which is surrounded by a lipid bilayer. It has been postulated that these vesicles respond to changing external environments by shape transformations, and this deformation enables them to release the drug across various barriers. Surfactants such as sodium deoxycholate are used to render these vesicles deformable. Conventional vesicles and deformable vesicles (with sodium deoxycholate) containing insulin were administered using a buccal spray to male rabbits and blood samples were taken. These data were compared to subcutaneous administration of insulin. The results showed that the entrapment efficiencies of the deformable and conventional vesicles were $18.87\% \pm 1.78\%$ and $22.07\% \pm 2.16\%$, respectively. The relative bioavailability of the insulin-deformable vesicles group was 19.78% as compared to subcutaneous administration. This bioavailability was found to be higher than that from conventional insulin vesicles.

9.7.5.4 Nanoparticles

In an effort to develop an effective bioadhesive system for buccal administration, insulin was encapsulated into polyacrylamide nanoparticles by the emulsion solvent evaporation method [98]. Though nanoparticle formation ensures even distribution of the drug, pelleting of the nanoparticles was performed to obtain three-dimensional structural conformity. In addition, it was hypothesized that the pelletized particles will remain adhered to the mucosa, leading to good absorption. While studying bioadhesion and drug release profiles, it was found that the

system showed a sustained drug release profile that was mainly governed by polymer concentration. A significant and nonfluctuating hypoglycemic response with this formulation was observed after 7 h in diabetic rats.

9.7.5.5 Microparticulate Delivery Systems

Microparticulate delivery systems containing piroxicam in amorphous form were designed to improve the drug dissolution rate via the sublingual route [99]. Two low-swellaible mucoadhesive methacrylic copolymers, namely Eudragit[®] L sodium salt (EuLNa) and Eudragit[®] S sodium salt (EuSNa), were chosen as carriers for the preparation of the microparticles. Two series of microparticles containing piroxicam and EuLNa or EuSNa in ratios ranging from 15:85 to 85:15 (m/m) were prepared by spray drying. The effect of the different compositions on the dissolution profile of piroxicam was determined. In addition, the mucoadhesive properties were also assessed. The microparticles of piroxicam and the copolymer improved the piroxicam dissolution rate in comparison with that of micronized piroxicam in cubic form. Also, the drug released from the microparticles reached a plateau within 12 min, and the concentrations were always higher than the maximum solubility of piroxicam in the cubic form.

9.8 IONTOPHORESIS

Iontophoresis is the process of delivering drugs or other charged molecules across a membrane using a small electrical charge. The “like-repels-like” phenomenon is applied here to drive charged molecules that are repelled by similarly charged electrodes into a tissue. Besides its use in transdermal delivery, this method has also been used to enhance oral mucosal drug delivery. Jacobsen [100] used iontophoresis to enhance the absorption of atenolol into porcine buccal mucosa. A newly designed *in vitro* three-chamber iontophoretic permeation cell was used to measure the permeability of the drug over a period of 8 h. High enhancement ratios were obtained, and were found to be a factor of the electric current rather than the concentration gradient.

Though this method can be used to increase the penetration of drugs, the inconvenience and accessibility issues faced in administration to the oral mucosa limit its applications.

9.9 BUCCAL AND SUBLINGUAL DELIVERY OF PEPTIDES AND PROTEINS

Proteins and peptides have emerged as an important class of therapeutic agents. The advances in biotechnology, proteomics, and increasing clinical applications have resulted in an increase in the number of formulations that are developed and introduced into the market. The buccal route has been researched for peptide delivery to overcome the disadvantages of the oral and parenteral routes. With oral delivery, peptides are quickly degraded in the GI tract since they are susceptible to degradation by the acidic pH of the stomach and metabolism by the peptidases present in the luminal, brush border, and cytosolic membranes. Also their large size, associated charge, and hydrophilicity hinder absorption through the intestinal epithelium. Most importantly, they undergo hepatic first-pass metabolism, which further reduces the bioavailability [101,102]. The parenteral route has also been extensively used for the delivery of peptides. This route, however, necessitates frequent injections to maintain therapeutically significant levels of the drugs due to short biological half-lives of the molecules leading to irritation at the site of delivery and reduced patient comfort and compliance.

In order to overcome these issues, various noninvasive routes are tested for the delivery of peptides. The oral mucosa due to its high vascularity, avoidance of hepatic first-pass metabolism, and the absence of degradative enzymes normally present in the GI tract has been explored as a suitable route for peptide delivery. Several studies of peptide absorption through the oral mucosa have been conducted, and the results have been impressive in some cases, and not in the others. The development of mucoadhesive systems for buccal and sublingual delivery has increased the absorption and bioavailability of peptides, and various formulations have been developed using these systems.

The factors that hinder the absorption of peptides through the intestinal epithelium, namely high molecular weight, charge, and hydrophilicity also affect their absorption through the oral mucosa. Combinations of mucoadhesive systems, absorption enhancers, and enzyme inhibitors have enabled better absorption.

A mucoadhesive buccal patch was evaluated for transmucosal delivery of oxytocin (OT) [103]. OT was incorporated with coformulations of Carbopol 974P and silicone polymer. The plasma concentrations of OT remained 20- to 28-fold greater than levels obtained from placebo patches for a period of 0.5 to 3.0 h.

Transmucosal delivery of salmon calcitonin (sCT) via the buccal route was studied using a mucoadhesive bilayer thin-film composite (TFC) [104]. *In vitro* studies showed that over 80% of sCT was released from the TFCs within 240 min. The relative bioavailability for rabbits treated with the film composites was $43.8\% \pm 10.9\%$ as compared to intravenous injection.

Buccal delivery for insulin has been investigated using different formulations such as buccoadhesive tablets [105], deformable vesicles [97], and pelleted bioadhesive polymeric nanoparticles [98].

Generex Biotechnology (Toronto, Ontario, Canada) markets a spray for delivery of insulin through the buccal mucosa [106]. The spray called Oralin, uses the RapidMist[®] technology, has been also developed by Generex Biotechnology. The device sprays a high-velocity, fine-particle aerosol into the patient's mouth, which results in an increased deposition of the particles over the mucosa. Since the particles are very fine and move fast, the insulin molecules delivered through this system traverse the topmost layers of the epithelial membrane, pass through the other layers, and are absorbed into the blood stream with the aid of absorption enhancers. Oralin has been found to produce rapid absorption and metabolic control comparable to subcutaneously injected insulin.

9.10 CONCLUSION

Despite various disadvantages, the oral mucosal route might be the potential option for drug delivery and for macro- and micromolecular deliveries. While buccal sprays, tablets, lozenges, and patches for smaller molecules have already been commercialized, not many buccal peptide formulations have been marketed. Administered peptides still remain susceptible to the permeability and enzymatic barrier of the buccal mucosa, and in many studies only moderate bioavailability has been observed. The advent of techniques like enzyme inhibitors, effervescent tablets, mucoadhesive devices, and absorption enhancers along with other advantages such as patient acceptability and low degradation have initiated numerous studies for delivery of proteins and peptides by this route. The development and evaluation of thiomers (thiolated polymers) for buccal delivery of peptides discussed earlier in this chapter provide many advantages in one system [86]. The applications of chemical enhancers as a promising technique for improved buccal and sublingual delivery are discussed in the next chapter.

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