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REVIEW ARTICLE

Critical evaluation of permeation enhancers for oral mucosal drug delivery

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Abstract

Background: Drug delivery via oral mucosa is an alternative method of systemic administration for various classes of therapeutic agents. Among the oral mucosae, buccal and sublingual mucosae are the primary focus for drug delivery. Buccal delivery offers a clear advantage over the peroral route by avoidance of intestinal and hepatic first-pass metabolism. However, despite offering the possibility of improved systemic drug delivery, buccal administration has been utilized for relatively few pharmaceutical products so far. One of the major limitations associated with buccal delivery is low permeation of therapeutic agents across the mucosa. Various substances have been explored as permeation enhancers to increase the flux/absorption of drugs through the mucosa, but irritation, membrane damage, and toxicity are always associated with them and limit their use. A clinically accepted permeation enhancer must increase membrane permeability without causing toxicity and permanent membrane damage. To date, the information available on oral mucosal permeation enhancement is much less than transdermal enhancement, though oral mucosa is more resistant to damage than other mucosal membranes. This article reviews the various categories of permeation enhancers for oral mucosal drug delivery, their mechanism of action, their usefulness, and the limitations associated with their use. *Conclusion*: To optimize the concentration of enhancer to limit its toxicity while facilitating an enhancing effect reproducibly will be a big challenge for future developments. Advances in permeability modulation and formulation with appropriate enhancers can provide for effective and feasible buccal drug delivery for many drugs, which otherwise have to be injected or ingested with water.

Key words: *Buccal; membrane damage; oral mucosal delivery; permeation enhancement; toxicity*

Introduction

In recent years, there has been tremendous interest and development in the field of mucosal drug delivery systems (such as ocular, nasal, pulmonary buccal, sublingual, rectal, and vaginal) as needle-free routes of administration for systemic drug delivery of therapeutic agents. Among various routes of drug delivery, oral route is perhaps the most preferred by the patient and clinician alike. Within the oral cavity, the two most useful mucosae for systemic delivery of drugs are buccal and sublingual membranes[1].

Even though sublingual mucosa is relatively more permeable than buccal mucosa, it is not a suitable site for oral mucosal delivery systems. The sublingual region lacks immobile mucosa and is constantly washed by considerable amount of saliva, making it difficult for dosage form retention. Sublingual region produces a rapid onset of action making it suitable only for highly permeable drugs with shorter delivery period requirements and an infrequent dosing regimen[2]. Because of these two important differences, buccal route is more preferred for systemic transmucosal drug delivery[1,3]. It is relatively permeable to many therapeutic agents, has rich blood supply, is readily accessible, robust, and exhibits fast cellular recovery following local stress and damage[4–7]. The virtual lack of Langerhan cells makes the oral mucosa tolerant to potential allergens[8]. Moreover, buccal drug delivery systems bypass first pass metabolism and avoid pre-systemic

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elimination in the GI tract. All these factors make buccal route a very attractive and feasible choice for sustained and controlled drug delivery systems[1,3] as rapid onset of action and dosage form retention is possible.

Oral mucosa: an overview

Anatomy and physiology

The structure and function of oral mucosa has been extensively discussed by various authors[1,3,9,10] (Table 1). According to Shojaei, oral mucosa is leaky epithelium intermediate between that of epidermis of skin and intestinal mucosa[2]. In terms of surface area, the oral mucosa has the minimum surface area (~200 cm^2) followed by skin (~20,000 cm^2) and then by gastrointestinal tract (~350,000 cm^2)[11]. As shown in Figure 1, it is made up of stratified squamous epithelium and connective tissue layer called lamina propria containing variable amounts of collagen, elastic fibers, and cells in aqueous ground substances[2,3]. The two layers are separated by basal lamina. Oral epithelia may be keratinized, para-keratinized, or nonkeratinized. The keratinized epithelia (gingival and hard palate) are relatively impermeable to water and show barrier function. Barrier function is because of the presence of neutral lipids such as ceramides and acylceramides. On the other hand, nonkeratinized epithelia (buccal, sublingual and soft palatal) do not contain acylceramides and only have small amounts of ceramides. They also contain small amounts of neutral but polar lipids, mainly cholesterol sulfate and glucosyl ceramides. These epithelia are found to be more permeable to water than keratinized one[9]. Keratinized and nonkeratinized tissues occupy about 50% and 30%, respectively, of the total surface area of the mouth[12]. The epithelia serves as a mechanical barrier, protecting underlying tissues, whereas the lamina propria acts as a mechanical support and also carries blood vessels and nerves. Underneath the lamina propria, a very loose submucosa (innermost layer) is present in the region from which the membrane originates.

The cheeks form lateral walls of the buccal cavity, which is covered by nonkeratinized stratified squamous epithelium. The hard palate, which is formed by roof bones, is covered by mucous membrane and forms a bony partition between the buccal cavity and the nasal cavity. The soft palate forms the back (muscular) portion of the roof and is also lined by mucous membrane. The sublingual mucosa, beneath the tongue, forms the floor of the mouth. Schematic representation of human oral cavity is shown in Figure 2.

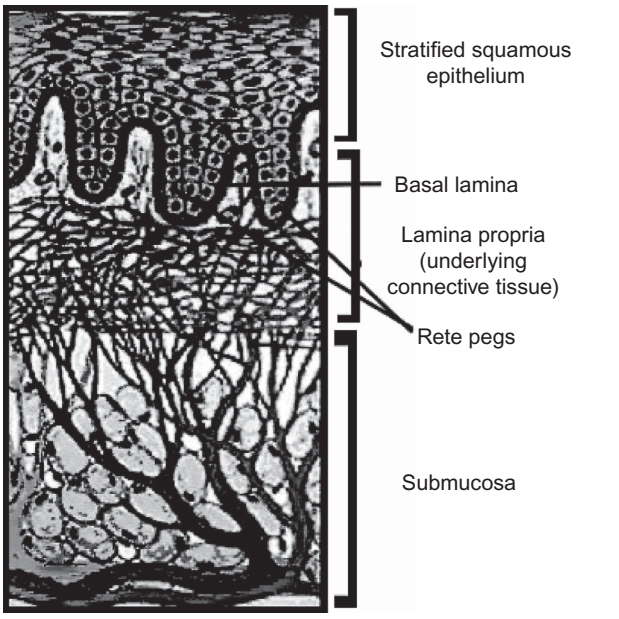

Figure 1. Main tissue components of oral mucosa (modified from Shojaei[2]).

Anatomical and physiological differences in oral mucosa are the main factors that cause discrepancy between humans and animals with respect to permeation of pharmacological agents. Although there is not much variation in the thickness of buccal mucosa in different species, there lies a major interspecies difference in terms of degree of keratinization and microflora in the buccal cavity (Table 2). Buccal mucosa of rabbit and dog has been claimed to be broadly similar in structure and composition to human buccal mucosa. On the other hand, the mucosa of rat and hamster is

Table 1. Essential features of oral mucosa in context to drug delivery.

Tissue and structure[195]	Thickness (μm)[195]	Turnover time (days)	Surface area[12] (cm^2) ± SD	Permeability[4]	Residence time[4]	Blood flow[196,a]
Buccal (NK)	500–600	5–7	50.2 ± 2.9	Intermediate	Intermediate	20.3
Floor of mouth—sublingual (NK)	100–200	20	26.5 ± 4.2	Very good	Poor	12.2
Gingival (K)	200	—	—	Poor	Intermediate	19.5
Palatal (K)	250	24	20.1 ± 1.9	Poor	Very good	7.0

NK, nonkeratinized; K, keratinized.
[a]In rhesus monkeys (mL/min/100 g tissue).

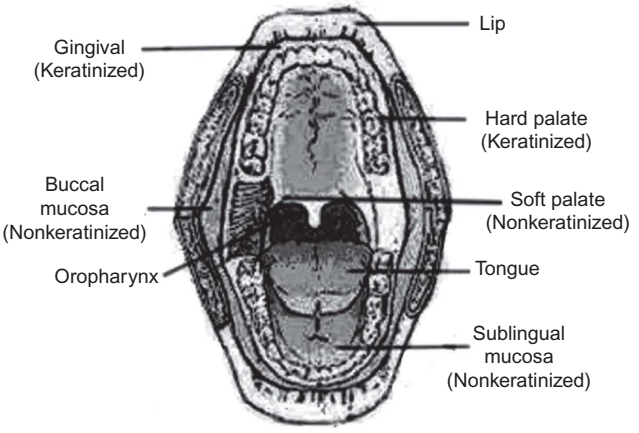

Figure 2. Schematic representation of human oral cavity (modified from Birudaraj et al.[194]).

keratinized, which makes these species unsuitable for research on oral mucosal drug delivery systems.

With respect to structure, the oral mucosa is divided into three types:

1. masticatory mucosa,
2. lining mucosa,
3. specialized mucosa.

Masticatory mucosa consists of keratinized stratified squamous epithelium and covers gingival and hard palate regions that are subject to mechanical forces of mastication, such as abrasion and stress. It closely resembles the epidermis of skin in its pattern of maturation.

Lining mucosa is nonkeratinized and can vary considerably in thickness in different oral regions. In general, it is thicker than masticatory mucosa and more permeable than the keratinized masticatory regions or skin[13]. This type of epithelium is found in buccal and sublingual regions and on the inner side of lips.

Specialized mucosa is found on the top surface of tongue and is of least importance to drug absorption. It has the characteristics of both masticatory and lining mucosa.

Various types of oral mucosa differ in their relative surface area in the oral cavity. Masticatory mucosa is approximately 25%, lining mucosa is approximately 60%, and specialized mucosa is approximately 15% of the total surface area of oral lining[12].

Permeability

There is a sizeable difference in the permeability characteristics between different regions of oral mucosa. Kurosaki and Kimura presented an excellent review on regional variation in oral mucosal drug permeability[14]. The permeability of oral mucosa decreases in the order of sublingual followed by buccal and then by palatal[1]. This rank order is based on the degree of keratinization and relative thickness of these tissues. Permeability is inversely proportional to the degree of keratinization and relative thickness. It is estimated that the permeability of the buccal mucosa is 4–4000 times greater than that of skin[15]. Small molecules (<75–100 Da) are able to cross the oral mucosa rapidly. There is an inverse relationship between permeability and molecular size.

Table 2. Comparison between buccal mucosa and salivary enzymes' activity of different mammals.

Part	Parameter	Dog	Hamster	Human	Pig	Rabbit	Rat
Buccal mucosa	Tissue structure[1]	NK	K	NK	NK	PK	K
	Mean thickness[197] (μm)	770	—	500–600	770	600	—
	Enzymes present[198]	E	—	AP, CP, D, E	AP, CP, E	AP, CP, P	CP, E
Saliva	Parotid enzyme activity[199]						
	β-D-Galactosidase	Moderate		Absent		Marked	Marked
	Acid phosphatase	Moderate		Marked		Marked	Marked
	Alkaline phosphatase	Absent		Absent		Absent	Intense
	Amylase	Absent		Intense		Intense	Intense
	Nonspecific esterase	Moderate		Weak		Moderate	Marked
	Pseudo-cholinesterase	Moderate		Weak		Absent	Marked
	Submandibular enzyme activity[199]						
	β-D-Galactosidase	Marked		Absent		Marked	
	Acid phosphatase	Moderate		Marked		Moderate	
	Alkaline phosphatase	Absent		Absent		Absent	
	Amylase	Absent		Intense		Absent	
	Nonspecific esterase	Marked		Moderate		Moderate	
	Pseudo-cholinesterase	Moderate		Weak		Absent	

NK, nonkeratinized; K, keratinized; PK, para-keratinized; AP, aminopeptidase; CP, carboxypeptidase; D, dehydrogenase; E, esterase; P, protease.

The permeability constant for human buccal mucosa for tritiated water was found out to be $579(\pm 122) \times 10^{-7}$ cm/min[13]. In case of oral mucosal drug absorption, the effect of molecular size on penetration is much less significant than partitioning and solubility. The permeability of ionizable drugs across buccal mucosa follows pH partition theory, characteristic of passive diffusion[16]. Increasing nonionized fraction of ionizable drugs could favor drug permeation through the transcellular route. Degree of ionization depends on the pH of the oral cavity and pK_a of the drug. So, control of pH in the oral cavity is critical for successful buccal delivery of ionizable drugs. The in vitro permeation of ondansetron HCl[17] and salbutamol sulfate[18] is pH dependent. Highest permeation was observed at those pH values where the drug remains preponderantly in the nonionized form. This is due to the fact that nonionized form possesses a high degree of lipid solubility and therefore shows a good affinity for the mucosal membrane of the oral cavity.

Permeability barriers to oral mucosal drug absorption

To pass through the oral mucosa, drug has to pass through different barriers present in the oral mucosa. There exists a lipophilic barrier (cell membrane), a hydrophilic barrier (interior of the cells), and an enzymatic barrier in passage of drug through the mucosa[11]. The oral mucosal drug permeation involves partitioning into and diffusion across each of the mucosal layers and any layer could potentially be a major barrier to drug penetration[19].

The permeability barrier to the oral mucosa is because of the presence of intercellular material derived from the so-called membrane-coated granules (MCGs)[2,3]. MCGs are spherical/oval granules 1–3 μm in diameter and are located in the intermediate cell layers of keratinized and nonkeratinized epithelia. When cells go through differentiation, MCGs start forming, they fuse with plasma membrane at the apical cell surfaces, and their contents are discharged into the intercellular spaces at upper one third of the epithelium. The components of MCGs in keratinized and nonkeratinized epithelia are different. The MCGs of keratinized epithelium consist of lamellar lipid stacks, glycoproteins, and number of hydrolytic enzymes whereas the nonkeratinized epithelium contains nonlamellar MCGs. The MCG lipids of keratinized epithelia include sphingomyelin glucosylceramides, ceramides, and other nonpolar lipids, whereas the major MCG lipid components for nonkeratinized epithelia include cholesterol, cholesterol esters, and glycosphingolipids[9].

Selvaratnam and his co-workers developed an in vitro model of human oral mucosa from oral keratinocyte cultures (obtained from healthy adults) and studied its permeability barrier properties. Cultures of human oral mucosal keratinocytes showed similar permeability properties and barrier lipid composition to their site of origin[20]. This system can be served as a useful model for the evaluation of local and systemic oral mucosal drug delivery.

Mucus layer and saliva

The secretion of saliva is affected by disease and various stimuli[21]. An acidic excipient can stimulate the secretion of saliva, which is an important aspect in selecting formulation excipients as an increase in salivary flow may decrease the permeability of the mucosa to certain drugs. There is a transitory decrease in water permeability through rabbit oral mucosa in vitro by the addition of human submandibular saliva to it[22]. The permeation of certain drugs through oral mucosal surface was increased by treating it with anticholinergic agents because it results in decreased salivary flow[23]. Saliva contains no proteases but moderate levels of esterases, carbohydrases, and phosphatases which may degrade certain drugs[24]. Although saliva secretion facilitates the drug dissolution process, involuntary swallowing of saliva may result in drug loss from the site of absorption[25]. Mucus layer, with thickness in the range of 1–400 μm, acts as a physical barrier to drug permeation through buccal mucosa[16] but can be advantageous for bioadhesive dosage forms. Short turnover time of mucus layer is sometimes detrimental to achieve long-term bioadhesion and sustained drug release. Drugs can interact with mucin through electrostatic attractions, hydrogen bonding, or hydrophobic interactions and can prevent their transport through the epithelia[26].

The barrier function of mucus layer was trivial when compared to other barriers that a drug comes across during its passage through the oral mucosa. This shows that the barrier function not only depends on the physicochemical properties of the drug but also on the physicochemical properties of the barrier and its relative thickness[19].

Keratinized layer

This layer acts as a major permeation barrier to some drugs. It shows reduced permeability because of the presence of complex mixture of lipids (glycolipids) in the intercellular spaces[9]. In different studies, the permeabilities of various compounds were determined through full-thickness and tape-stripped hamster cheek pouch mucosa[27,28]. The compounds were found to be more permeable through tape-stripped tissue, and it was concluded that keratinized layer of hamster cheek pouch poses a major barrier to the transport of these compounds through the mucosa.

Outer epithelium

The outer epithelium is considered to be the rate-limiting membrane to mucosal permeation. This barrier exists in the outermost (200 μm) superficial layer of the oral mucosa (i.e., uppermost 20–30% of epithelial layer)[29,30]. Squier and his co-workers performed a series of permeation studies using large molecular weight tracers, such as lanthanum nitrate[31,32] and horseradish peroxidase[33,34] to understand the barrier functions of epithelium. When applied to the outer surface of epithelium, these tracers penetrate only through the outermost two or three layers of cells. When applied to the subepithelial region, they permeate through the connective tissue, the basal lamina, and through the lower 75% of intercellular spaces of the epithelium but not into the outermost 25% cell layers of the epithelium. This suggested that flattened surface cell layers, which are present in upper one third of the epithelium, present the main barrier to permeation, whereas the more isodiametric cell layers are relatively permeable.

Basement membrane or basal lamina

The basement membrane is a continuous membrane having thickness of approximately 1 μm. It also presents some hindrance to permeation of proteins, immune complexes, endotoxins, and certain therapeutic agents such as chlorhexidine and beta blockers[19].

Lamina propria

Its structure is not dense enough to exclude relatively large molecules and therefore it is readily permeable to hydrophilic compounds and large molecules[1].

Pathways of drug absorption through buccal mucosa

There are two major pathways for drug permeation across the oral mucosa as shown in Figure 3. Drug diffusing through or partitioning across the membrane of oral cavity follows the path of least hindrance. As the oral epithelium is stratified, the drug molecules may follow one route in one region and the other route in other region[19]. These two routes are as follows:

- *Transcellular pathway.* This is intracellular route where compounds transverse the epithelium across the cells. Permeability of the drugs transported by this route is dependent on their partition coefficients.
- *Paracellular pathway.* This is intercellular route where the compounds diffuse through the intercellular lipids between the cells[35,36]. Permeability of the drugs transported via this route is independent of their partition coefficients.

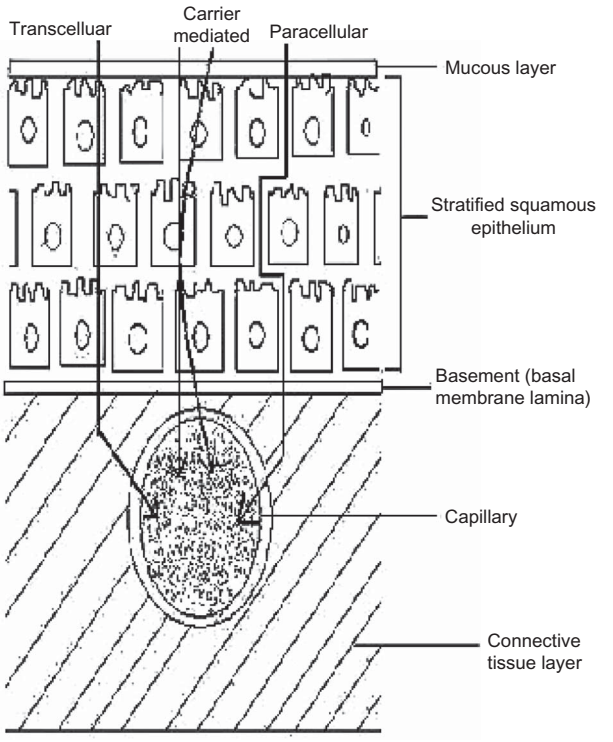

Figure 3. Possible routes of drug transport through oral mucosa (modified from Rathbone and Tucker[19] and Senel and Hincal[77]).

Permeants (lipid-soluble and water-soluble) can penetrate the epithelium by either route, but one route is usually preferred over the other depending on the physicochemical properties of the diffusing substance, composition and organization of the phases encountered during penetration process, and the properties of a penetration enhancer, if used. The intercellular spaces and cytoplasm are hydrophilic in nature and therefore lipophilic compounds would have low solubilities in this environment. The cell membrane is rather lipophilic, and hydrophilic solutes will have difficulty permeating through the cell membrane because of a low partition coefficient. So, intercellular spaces pose a major barrier to the permeation of lipophilic compounds, and the cell membrane acts as a major transport barrier for hydrophilic compounds[2]. Siegel and Gordan studied the permeation of 20 compounds across oral mucosa in vivo. The permeability constants (K_P) of straight chain lipophilic compounds were proportional to their oil-in-water partition coefficients (K_w). Introduction of –OH groups to the straight chain resulted in increased hydrogen bonding and hence decreased K_P. The K_P values of hydrophilic compounds were found to be greater than would be expected from their K_w values[37].

Transcellular pathway

Although surface area available for the absorption is considerably greater for the intracellular route, this path offers considerable diffusional resistance because the drug has to alternatively cross the hydrophilic and lipophilic phases of epithelial cells. There are reports in the literature suggesting that drugs penetrate oral epithelium via the intracellular route. The hydrophilic drugs cross this route through aqueous pores in the plasma membrane of epithelial cells via a specialized transport mechanism[38]. The lipophilic drugs penetrate this route through the lipid bilayer of plasma membrane. The flux of the drug (J_T) in transcellular route can be given as follows[39]:

$$J_T = (1-\varepsilon)\frac{D_T \times K_T \times C_D}{h_T}, \quad (1)$$

where ε is the fraction of the surface area of the transcellular route, D_T is a diffusion coefficient in the intracellular spaces, h_T is the path length of transcellular route, C_D is the concentration of the drug at donor site, K_T is the partition coefficient between lipophilic region (cell membrane) and hydrophilic region (intercellular space and cytoplasm). Equation (1) indicates that drug flux through transcellular route is directly proportional to the partition coefficient.

There are various evidences reported for this route. Beckett and Moffat studied the oral mucosal absorption of various amines and acids. They found that permeability of these agents through the mucosa was proportional to their partition coefficients. Le Brun et al. investigated the in vitro penetration of some beta-adrenoreceptor-blocking agents through porcine buccal mucosa. The transport of these agents was found to be dependent on their partition coefficients[40]. Garren and Repta reported similar partition coefficient-dependent transport for substituted acetanilides[28]. Siegel et al. determined the permeability of various organic solutes and the number of structurally unrelated compounds through rabbit's and canine's lingual frenula and rat's tongue. They proposed that these compounds cross the oral mucosa via pores in the membrane and their transport depends on the partition coefficient of these agents[41]. All these studies suggested that the transcellular route was involved in the drug transport across the mucosa. Zhang et al. studied the transport pathway of fentanyl by exploring its accumulation inside the oral mucosa of dogs. It was seen that the accumulation of drug in the mucosa is pH dependent and the transport route is transcellular[42].

Paracellular pathway

This route involves passage between the cells through lipid material of the intercellular spaces. This pathway is very complex, requiring the epithelium to have a sufficiently open matrix and drug to have a considerable affinity for, and diffusivity in, the intercellular fluids[1]. Lipid-soluble drugs cross by diffusion through the lipid of intercellular matrix while water-soluble drugs pass via aqueous channels in the intercellular spaces of the epithelium. These drugs may follow this route even in their unionized forms. The area available for transfer is relatively small in comparison to the intracellular route. However, it is the predominant route for most of the pharmacologically active agents. Flux of the drug (J_P) in paracellular route can be written as follows[39]:

$$J_P = \varepsilon\frac{D_P \times C_D}{h_p}, \quad (2)$$

where ε is fraction of the surface area of paracellular route, D_P is the diffusion coefficient in the intercellular spaces, h_P is the path length of paracellular route.

In literature, there are various evidences for this route[19]. Following topical or subepithelial administration of lanthanum to the oral mucosa of rabbit and rat, the electron-dense tracer compound was located in intercellular spaces[31]. Hill and Squier found that the horseradish peroxidase and lanthanum took intercellular spaces as the major route of transport through neonatal palatal mucosa maintained in organ culture for a period of up to 24 days[32]. Horseradish peroxidase was viewed in the intercellular spaces of oral tissue excised from rabbit, monkey, and rat following topical or subepithelial administration of the compound. Dowty et al. studied the transport of thyrotropin-releasing hormone (TRH) across rabbit buccal mucosa. They found that the transport is independent of pH and it primarily follows intercellular pathway to cross the mucosa[29]. They also found that by reducing the intercellular permeation barrier, penetration of horseradish peroxidase was increased through intercellular regions[35]. Squier and Lesch studied the penetration pathways of radiolabeled cholesterol, ethanol, and water across porcine buccal mucosa in vitro. Autoradiography studies revealed that intercellular route was the predominant route of transport of these agents across the superficial layers of buccal mucosa[43]. Zhang et al. investigated the transbuccal permeability of isoproterenol using an in vivo dog model. They reported that the permeation was independent of pH of the donor side and the transport route was paracellular pathway[44]. Hoogstrate and co-workers studied the absorption of fluorescein isothiocynate (FITC)-labeled dextran (molecular weight,

10 kDa) through porcine buccal mucosa and found that dextran penetrates the tissue via intracellular route. Even 4-kDa dextran follows same route for its absorption through the mucosa[45].

Specialized transport mechanisms

Although passive diffusion is the major transport mechanism for drug permeation across buccal mucosa, there is an existence of specialized transport mechanism(s), such as involvement of carrier systems, for the transport of certain drugs and nutrients through oral cavity. The absorption of D-glucose and L-arabinose across buccal mucosa was shown to be both saturable and stereospecific[38]. This indicated the presence of a carrier-mediated transport system for these sugars, as saturation and stereospecificity are not characteristics of passive diffusion process. Such a specialized mechanism for D-glucose transport was also observed in a cultured stratified cell layer of human oral mucosal cells[46]. In addition to sugars, absorption of various vitamins, including L-ascorbic acid, nicotinic acid, and nicotinamide, have been shown to be dependent on the presence of sodium ions, indicating absorption from the oral cavity by carrier-mediated processes[47,48].

Utoguchi et al. proposed that monocarboxylic acids undergo a carrier-mediated transport from cultured oral mucosal epithelial cells of rabbit buccal mucosa[49]. Similar mechanism was proposed for the absorption of salicylic acid from hamster cheek pouch mucosa in vivo[50]. Kurosaki et al. suggested the presence of specialized transport mechanism for the transport of cefadroxil and D-glucose across human buccal cavity and dorsum of tongue, respectively[36,51].

There has also been a report regarding the active transport of antibacterial agents in oral mucosa. In a cell line derived from oral epithelium, the uptake of ciprofloxacin and minocycline was not only saturable and inhibited in the presence of other compounds, but the intracellular levels of both antibiotics were 8- to 40-fold higher than the extracellular levels, demonstrating an active transport process[52].

Methods used to evaluate mucosal permeation

In vitro methods

Isolated mucosal tissue mounted in permeability chambers

The most widely used in vitro methodology to study oral mucosal permeability across the mucosa is by testing drug permeation across isolated mucosal tissue mounted in permeability chambers. Two types of permeability cells have been used: side-by-side horizontal (Ussing chambers) and vertical (Franz diffusion cell) cells. The buccal mucosa of monkeys, dogs, and pigs has been extensively used for in vitro drug permeability studies. The porcine buccal tissue is the most frequently utilized model because of relative similarity to human tissue and a low cost associated with tissue acquisition.

Advantages

- Diffusion cells are very useful to measure the transmembrane flux of a substance across a mucosa and to study the effects of absorption enhancers on the membrane.
- These cells are also easily adapted to measure transepithelial electrical resistance and thus monitor the integrity of the membrane.
- They are also useful for physiological and toxicological studies.
- Experiment can be performed in controlled environment under well-defined conditions.

Disadvantages

- Limited surface area, presence of tissue damage from mastication, laborious and time-consuming excision procedure, minimal potential for assay automatization, and large variability in permeation results among replicates.
- Tissue is removed from its natural environment and placed in a highly artificial environment. Therefore, tissue viability is a concern.
- Tissue used in permeation studies should be fresh because freezing and thawing may alter the permeability properties of oral mucosa.
- Difficult to verify that the permeability characteristics of isolated tissue are identical to those of the living animal.
- Determination of statistically significant differences in permeation for compounds that have relatively close rates of buccal permeation may be difficult in diffusion cells because of large interanimal variability and it may require a large number of animal tissue samples.

Buccal epithelial cell cultures

Because of the drawbacks of tissue models, attempts have been made to establish suitable in vitro buccal epithelial cell culture models for testing drug permeation and metabolism. Tavkoli-Saberi and Audus developed the hamster pouch epithelium culture. They demonstrated that this cultured epithelial membrane has similar permeability properties to freshly excised tissue[53]. In addition, a TR146 cell culture derived from human buccal metastasis has been used to assess drug transport and

metabolism[54,55]. Recently, a new cultured human buccal epithelium model EpiOral™ has become commercially available (MatTek, Ashland, MA, USA). This tissue model consists of normal human keratinocytes cultured to form a three-dimensional differentiated tissue that histologically and biochemically resembles human buccal tissue[56]. So far, this model has shown good correspondence to human buccal mucosa in terms of structure, protein expression, and lipid content with good reproducibility of the tissue cultures[57]. Also, a good correlation between the permeation of fentanyl across cultured buccal epithelium, applied in different tablet formulations, and bioavailability in humans has been found[58,59,60].

Advantages

- It simplifies the experimental procedure of using excised animal mucosa and provides an alternative to the time-consuming and more expensive animal studies.
- Offers the advantages of homogeneity of cell type, ease of handling, ability to study transport under controlled conditions, and manipulation of parameters influencing transport.
- It is very useful in investigating drug trafficking at a cellular or subcellular level as well as specific drug transport mechanisms. It may provide the opportunity to use human rather than animal tissues.

Disadvantages

- Cell culture models tend to be less suitable than isolated mucosal tissue for excipient testing because cultured cells are more susceptible to formulation excipient effects on cell viability and functional integrity.
- The effect of parameters that can affect drug transport, such as the control of culture growth rate, number of differentiated cell layers, lipid composition, and optimum day(s) in culture, is still not well characterized in these models.
- A number of factors, such as whether the cells are a primary culture or a stable cell line, whether they are normal or transformed, the differentiation potential of the cells, their passage number, cell heterogeneity, cell viability, and their phenotypic stability need to be considered in order to compare the morphological, biochemical, and transport properties of an in vitro cell culture model with the equivalent in vivo model.

In vivo methods

Buccal absorption test

The easiest and simplest intact mucosal permeability studies utilized biological response of the living organism locally or systemically to assess mucosal permeability[61]. One of the simplest measurements of penetration through oral mucosal tissue is 'buccal absorption test', also called as the 'swirl and spit test'. It was first introduced in 1967 by Beckett and Triffs[62] and has been widely utilized to determine drug absorption via the oral mucosa[63–67]. In this method, a buffered drug solution, of known volume and concentration, was placed in the subject's mouth and was swirled in the mouth for a defined length of time. The solution was then expelled and assayed. The amount of drug absorbed was determined by the difference between the amounts of drug introduced and recovered. Drug excipient interactions were also studied by this general test[68,69]. The researchers modified this test in several ways depending on the outcome required[70–72].

Advantages

- It allows drug disappearance from the mouth and appearance in the plasma to be determined simultaneously.
- Experiments are relatively straightforward, easy to perform, and permit collection of quantitative data directly from human subject.

Disadvantages

- The percent absorption should be substantial to get reliable data.
- Detailed knowledge of the drug's pharmacodynamics is required for the estimation of absorption from pharmacological data.
- Test cannot provide information as to the relative permeability of different regions of the oral cavity because complete oral mucosa is in contact with the drug solution.

Perfusion studies

A number of in vivo perfusion studies have been reported as a modification of the buccal absorption test[42,44,73–75]. These experiments were performed by attaching the perfusion chambers to the oral mucosa of anesthetized animals. Drug solutions were circulated through the device and collected at different time intervals. The chamber can be applied to various sites in the oral cavity and therefore experiment can be performed at several sites on the oral mucosa membrane at the same time. This method is commonly used in pharmacokinetic studies.

Disadvantages

- This approach is only useful for drugs whose biological effects can be measured, and their biological effects must be proportional to drug concentration.
- Local drug metabolism is the major problem that needs to be considered when this experiment is performed.

Need for enhancers

There are certain therapeutic agents that are easily permeated through oral mucosa. These agents, if given through mucosal route (buccal/sublingual), showed improved bioavailability as they bypass hepatic first pass metabolism and GI metabolizing enzymes. Nitroglycerine and isosorbide dinitrate are successfully delivered to sublingual mucosa, and their respective sublingual formulations are running successfully worldwide. But there are certain compounds that pose problems in penetrating the oral mucosal membrane. It is not necessary that the compounds having very good oral absorption also have good permeation through the oral mucosa. There are two main reasons for it:

- Difference in permeability characteristics of both the membranes: Oral mucosal membrane is less permeable than intestinal membrane. In a series of in vitro permeability studies with excised rabbit membranes, buccal mucosa was at least fivefold less permeable to carboxyfluorescein and showed fivefold lower electrical conductance than intestinal membrane[76].
- Difference in surface area of both the membranes: Oral mucosa has much smaller surface area available for absorption of buccal/sublingual dose than the intestinal membrane surface area.

Oral delivery of proteins and peptides is not feasible because of their rapid degradation by GI and hepatic proteolytic enzymes. So, there is specific interest in delivering proteins and peptides through the oral mucosal routes to achieve desired bioavailability and pharmacological activity by a noninjection route. Many peptides show poor permeation properties across the mucosal membranes and some show very slow diffusion through it. Apart from poor permeation and slow diffusion, various peptidases and proteases in the oral cavity could also contribute to the poor bioavailability. But poor membrane permeation is the main reason for the low bioavailability of these agents via the buccal route. There are evidences in the literature that certain hormones like TRH, oxytocin, and heparin exhibit poor absorption behavior when administered via sublingual or buccal route. The membrane of oral cavity exhibits barrier function, which limits the absorption of proteins and peptides through it. The problems associated with poor permeation and slow penetration of therapeutic agents across the mucosa can be overcome by the use of permeation enhancers.

Buccal permeation enhancers

These are the substances that promote drug permeation rate through the buccal mucosa[77]. They are often referred to as absorption enhancers/penetration enhancers/bioenhancers. The ideal enhancer should be safe, nontoxic, nonirritant, nonallergic, and chemically and pharmacologically inert[78]. Apart from that it should not alter the membrane properties permanently.

The goal of designing penetration enhancers, with improved efficacy and reduced toxicity profile, will be more readily realized by a thorough understanding of the relationship between enhancer structure and the effect induced in the membrane and, of course, the mechanism of action.

Enhancer efficacy depends on the physicochemical properties of drug, administration site, nature of the vehicle, and whether enhancer is used alone or in combination. Differences in cellular morphology, membrane thickness, enzymatic activity, lipid composition, and potential protein interactions are structural and functional factors that vary the effectiveness of the enhancer from site to site[79,80]. Permeation through buccal mucosa is considered to be a passive diffusion process[81–84] and is described well by Fick's law of diffusion.

$$J_{SS} = \frac{D \times K}{h} C_D, \tag{3}$$

where J_{SS} is the steady-state flux, D is the diffusivity, K is partition coefficient between the mucosa and the donor chamber, and h is the path length through which the drug must traverse (transcellular or paracellular). Permeation of drugs across buccal mucosa may be increased by increasing the diffusivity through the tissue (D), partitioning through the tissue (K), or the concentration of permeant at the mucosal surface. Permeation enhancers may improve the overall flux of a compound across a biological membrane by increasing any one or all of these factors[85].

Classification of mucosal permeation enhancers

Permeation enhancers can be classified into many categories depending upon their chemistry, mechanism of action, and the type of drugs with which they are used. The detailed chemical classification is tabulated in Table 3.

Surfactants and bile salts

Surfactants and bile salts have been extensively employed to enhance the absorption of drugs through various epithelia including buccal membranes[77,86]. It is well known that bile salts play an important role as physiological surfactants in the absorption of lipids and lipid-soluble vitamins. The widespread use of surfactants in pharmaceutical and cosmetic formulations has prompted considerable interest in the influence of

Table 3. Classification of mucosal permeation enhancers.

Class	Examples
Synthetic surfactants	*Cationic:* cetyl trimethylammonium bromide (CTAB), cetyl pyridinium chloride (CPC), benzalkonium chloride (BC)
	Anionic: sodium dodecyl sulfate (SDS), sodium laurate
	Nonionic: Laureth 9, dodecylmaltoside, Tween, Span, Brij, Myrj, polaxamer, sucrose esters
Bile salts and other steroidal detergents	Sodium cholate (SC), sodium deoxycholate (SDC), sodium glycocholate (SGC), sodium glycodeoxycholate (SGDC), sodium taurocholate (STC), sodium taurodeoxycholate (STDC), sodium tauroglycocholate (STGC), sodium glycodihydrofusidate (SGDHF), sodium taurodihydrofusidate (STDHF), sodium ursocholate (SUC), saponins
Fatty acids and derivatives	Lauric acid, oleic acid, caprylic acid, neodecanoic acid, elaidic acid
Phospholipids	Phosphatidylcholine (PC), lysophosphatidylcholine (LPC), didecanoylphosphatidylcholine (DDPC)
Chelators	Na_2EDTA, citric acid (sodium citrate), sodium salicylate (SS), methoxy salicylates (MS), polyacrylates
Cyclodextrins	α-, β-, γ-cyclodextrin, hydroxypropyl-β-cyclodextrin (HP-β-CD), methylated-β-cyclodextrin (M-β-CD)
Positively charged polymers	Chitosan, trimethyl chitosan, chitosan-4-thioglycolic acid, chitosan-4-thio-butylamidine, dextran sulfate
Positively charged polymers	Poly-L-arginine, L-lysine
Miscellaneous	Menthol, azone (AZ), aprotinin, alkyl glycosides, sulfoxides, unsaturated cyclic urea, polycarbophil, ethanol, etc.

these agents on drug absorption. They can have both enhancement and inhibitory effects on drug absorption. They can exert the following effects:

- Interaction with biological membrane, thereby modifying membrane permeability.
- Interaction with dosage form.
- Interaction with the drug. Surfactants enhance drug dissolution and solubilize fat-soluble components.

The various mechanisms proposed for surfactants and bile salts include extraction of lipid components of mucosa, denaturation, or extraction of proteins, enzyme interaction, swelling of tissues, membrane fluidization, and reverse micellization in the membrane[80,87]. The effects of surfactants on biological membrane are concentration and time dependent. Larger tissue perturbation is expected when either the concentration or the contact time is increased. At low concentrations, surfactants enhance the permeability of only those compounds that traverse the buccal mucosa via paracellular route. This was demonstrated by in vitro buccal permeability study of caffeine (CAF) and estradiol (E2) through porcine buccal mucosa using sodium dodecyl sulfate (SDS). SDS (0.05–1.0%, w/v) enhanced the permeation of CAF (hydrophilic) through paracellular route by solubilization of intercellular lipids, which act as rate-limiting barrier for paracellular permeants. On the other hand, SDS showed negative effect on E2 permeability because being a water-insoluble lipophilic molecule it would be expected to permeate buccal mucosa by transcellular route[88]. In another study, sodium glycocholate (SGC) was shown to enhance the buccal permeability of hydrophilic flecainide acetate and not the more lipophilic flecainide base, which was attributed to the different pathways for each permeant and the ability of SGC to affect only paracellular route[89].

However, at very high concentrations of surfactants and bile salts, both paracellular and transcellular routes are affected. Such an observation was made on the basis of confocal laser scanning microscopy to visualize various fluorescently labeled dextrans in the porcine buccal mucosa in the presence and absence of bile salts. At low concentrations of bile salt, amount of dextran present in the intercellular spaces was increased, suggesting that bile salt possibly solubilize the intercellular lipids and thus enhanced dextran permeability through paracellular route. At higher concentrations of bile salt, dextran appears in the epithelial cells, indicating that at higher concentration, bile salt was able to increase the permeability across the cell membranes because of disruption of cell membrane lipids[90].

Apart from extraction of mucosal lipids, bile salts such as sodium deoxycholate (SDC) and SDS also interact with proteinaceous domains of the tissue and affect the thermal transitions associated with tissue proteins and lipoproteins. This was associated with increase in salicylic acid permeability through excised rabbit buccal mucosa and a reduction in the electrical resistance of the tissue. SDC and SDS caused uncoiling and extension of protein helices, thereby increasing drug permeability through paracellular route[30].

The increase in membrane permeability has been related to surfactant irritative potential. Typically, ionic surfactants are more potent enhancers than nonionic compounds. But they are also considered to be more toxic because they can damage the permeability barrier at relatively low concentrations. They can also cause swelling of the buccal mucosa. SDS has been reported to increase buccal delivery of several drugs when included in dosage forms. However, it was reported to cause marked irritation to the buccal epithelium[16].

It has been observed that bile salts such as sodium glycodeoxycholate (SGDC) and SDS enhance the

permeation through the mucosa only when used above their critical micelle concentration (CMC)[88,91]. Sodium taurocholate (STC) and SGC enhanced the mannitol permeation across TR 146 cells when used in concentrations two- to three-fold greater than their CMC values[54]. At concentrations exceeding the CMC, these agents extract lipids from the mucosal surface and thus reduce barrier properties of the mucosa, resulting in enhanced drug permeability.

Bile salts also have inhibitory effects on mucosal membrane peptidases. It is generally considered that buccal mucosal damage caused by bile salts would be reversible and less serious because of the nature of buccal mucosa, but long-term safety studies have not been documented, which limits their use at higher concentrations.

Generex Biotechnology Corporation (Toronto, Canada) has developed a proprietary platform technology for precise insulin delivery. This novel, pain-free, oral insulin formulation has a critical series of attributes: rapid absorption, a simple (user friendly) administration technique, precise dosing control (comparable to injection within one unit), and bolus delivery of drug. The technology is based on a liquid formulation (*Oral-lyn*™) containing human recombinant insulin and a combination of absorption enhancers, such as surfactants (which not only allows the drug to traverse mucous membranes rapidly, but it also increases both the solubility and the stability of the drug) and the *RapidMist*™ device (metered dose inhaler). The system introduces a fine-particle aerosol at high velocity into the patient's breath; the mouth deposition is dramatically increased compared with conventional technology. No insulin is deposited into the lungs by the delivery system. The absorption enhancers coat the insulin and form micelles that are too large to enter the pulmonary tract, and absorption takes place in the inner cheek wall. The mixed micelles containing insulin molecules transverse the superficial layers of oropharyngeal mucosa and, with the aid of absorption enhancers, insulin is rapidly absorbed through the buccal mucosal lining, into the blood stream[92–94].

Fatty acids

Fatty acids are the most abundant lipids in the biological membranes. The use of fatty acids as skin penetration enhancers has been well established in the literature, but there are very few reports that demonstrate their use as buccal permeation enhancers. Administration of fatty acids, mainly of the *cis*-unsaturated variety (such as *cis*-9- and *cis*-11-octadecenoic acids), has been reported to increase membrane permeability by disruption of intercellular lipid packing and increasing fluidity. Medium chain fatty acids (C-6 to C-14) have been shown to be more effective than longer (<C-14) and shorter (C-6 to C-10) fatty acid chains[95]. It has been shown that the magnitude of enhancement is influenced by the presence and position of double bonds, isomer type (*cis*- or *trans*-), ionization state, chain length, degree of branching, nature of the vehicle used, fatty acid concentration, and the contact time. It is generally accepted that unsaturated fatty acids are more disruptive than saturated fatty acids with same carbon number. Inclusion of fatty acids in a membrane may change the van der Waals interactions between hydrocarbon chains and forms hydrogen bonds between fatty acid carboxyl groups and neighboring moieties[96,97]. Purified oleic acid was reported to modify the barrier property of rat buccal mucosa and thus led to remarkable and continuous hypoglycemic effect after buccal delivery of insulin from pluronic gels[98]. The possible mechanism could be the disruption of lipid bilayers in buccal mucosa although it has not been proposed by the authors. Permeation enhancement of lidocaine hydrochloride (LH), propranolol, and ergotamine tartarate has also been reported in the presence of oleic acid[99]. It was assumed that the enhancing effect was because of the lipid-fluidizing effects of oleic acid.

Birudaraj et al. investigated the transport of a lipophilic drug, buspirone, across porcine buccal mucosa in vitro. It was found that combination of oleic acid (5%) and propylene glycol (PG) (40%) in buffer resulted in the greatest flux of drug across mucosa. Transcellular pathway was found to be the major route of transport at neutral pH[100].

Tsutsumi et al. evaluated the effect of various permeation enhancers on transbuccal delivery of ergotamine tartarate through hamster cheek pouch in vitro. Cod-liver oil extract (CLOE), polyoxyethylene-hydrogenated castor oil, sodium caprate, and SGC were selected as permeation enhancers. Among the enhancers used, CLOE, which is a mixture of 16 types of unsaturated and saturated fatty acids and contains oleic acid as major component, showed greatest enhancement effect on ergotamine tartarate through hamster cheek pouch. The greatest enhancing activity of CLOE was seen at 5%; however, a further increase in the CLOE concentration from 7% to 10% resulted in a decreased enhancing activity[101]. Further studies proved that CLOE mainly enhanced the permeation of nonionized form of the drug. The possible mechanism could be distribution of the drug in the lipid-rich region of the buccal membrane and the resultant modification of barrier structure. This would enhance the permeability of nonionized drug via lipophilic route[102]. The CLOE showed similar enhancing effects through buccal mucosa of guinea pigs[103].

Fatty acid esters such as glyceryl monosterate, sodium laurate, sodium fusidate, sodium caprate, and sucrose fatty acid esters were also investigated as buccal permeation enhancers. These agents were less irritating

than bile salts and surfactants to buccal mucosa. Diethylene glycol monoethyl ether (transcutol) enhanced the permeation of essential oil components of Salvia desoleana through porcine buccal mucosa from microemulsion gels[104].

Ganem-Quintanar et al. studied the ex vivo oral mucosal permeation of LH with sucrose fatty acid esters as absorption enhancers. Among the sucrose esters studied, sucrose laurate enhanced buccal permeation of LH with a 22-fold increase in the enhancement ratio[105]. Other sucrose esters did not show any enhancing effect.

Einarsson reported the use of glyceryl esters of fatty acids to facilitate the mucosal absorption of heparin and their derivatives[106].

Lee and Kellaway reported the buccal permeation enhancement of [D-Ala^2, D-Leu^5] enkephalin (DADLE) across excised buccal porcine by glyceryl monooleate, which is a polar and sparingly water-soluble lipid and can form lyotropic liquid crystalline phases in the presence of water. Its cubic and lamellar liquid crystalline phases showed bioadhesive properties. The cubic phase also showed protective action against enzymatic degradation of peptide drugs and improved chemical stability of compounds containing amide groups. A co-transport mechanism of lipid and hydrophilic peptide was responsible for permeation enhancement. It is a promising drug carrier for the buccal delivery of peptide drugs as well as a penetration enhancer[107]. Authors also investigated the ex vivo buccal permeation of DADLE by incorporating oleic acid together with polyethylene glycol 200 (PEG 200) as a co-enhancer into cubic liquid crystalline phase of glyceryl monooleate. Buccal permeation was significantly increased when 5% or 10% of PEG 200 was co-administered with 1% oleic acid compared with the cubic phase containing 1% oleic acid alone. PEG 200 increased the aqueous solubility of oleic acid incorporated into the cubic phase and hence promoted the transport of oleic acid into buccal mucosa[108].

Burgalassi et al. investigated the effect of cetylpyridinium chloride (CPC), polyethoxylated castor oil (PCO), and PEG dodecyl ether on permeation of lorazepam from hydrophilic matrices using three models: (1) cultured monolayer of human buccal epithelial cells, (2) hamster cheek pouch mucosa in vitro, and (3) buccal administration to rabbits in vivo. In first two models the presence of enhancers (except when present at the higher concentrations) increased drug permeation rate, whereas in the in vivo model, only CPC was moderately active at the highest tested concentration, other two were inactive in vivo[109]. Different ex vivo and in vivo effects of the enhancers were partially explained on the basis of the structural characteristics of the absorbing membranes.

Hague and Stanley reported that bile salts are good enhancers for hydrophilic drugs whereas long chain fatty acids, and their analogs are more suited to lipophilic drugs. Synthetic surfactants like SDS and medium chain fatty acids (C-8 to C-14) and their derivatives can be used for both lipophilic and hydrophilic drugs[110,111].

Chitosan and derivatives

Chitosan exhibits several favorable properties such as biodegradability, biocompatibility, bioadhesion, antifungal and antimicrobial properties and attracts considerable interest in buccal delivery of antimicrobial agents[112]. In recent years, the absorption-enhancing effects of chitosan and its derivatives have been studied extensively. It has been proved that these compounds are potential absorption enhancers for mucosal delivery systems[113]. Chitosan, being a bioadhesive, has been shown to prolong retention times in oral mucosa when formulated as hydrogels[114]. Illum et al. first reported the permeation-enhancing effect of chitosan on transmucosal absorption of small polar molecules as well as peptides and proteins across nasal epithelium[115]. Atrusson et al. found that chitosan can also increase the paracellular permeability of ^{14}C-mannitol, which is a marker compound for paracellular routes, across CaCo-2 intestinal epithelial cells[116].

The main mechanism of intestinal permeation enhancement has been attributed to binding of the polymer to epithelial membrane through a charge-dependent effect that results in opening of tight junctions in the paracellular route[117]. The same mechanism is unlikely in case of buccal permeation enhancement as tight junctions are rare in buccal epithelium and are not solely responsible for barrier function[1].

Chitosan was also observed to have a significant enhancing effect on permeation of various therapeutic agents and peptides across the buccal mucosa. Two compounds, hydrocortisone and transforming growth factor-β, were formulated in gel form (2% chitosan-H gels prepared in dilute lactic acid) and applied on the surface of porcine buccal mucosa in vitro. Drug in phosphate buffer solution was used as control. In vitro flux of both compounds was significantly increased and high concentration of hydrocortisone was observed in the upper epithelial layer with chitosan gels compared to those with phosphate buffer solutions. There was less penetration of hydrocortisone into the deeper tissue layer because of its hydrophobicity, whereas hydrophilic peptide appeared in the deeper layers. Hence, chitosan increased the quantity of these compounds in superficial layer of the epithelium as compared to phosphate buffer solution. This effect may be related to both the direct fluidizing effect on organized intercellular lipid lamellae and bioadhesive nature of chitosan[118,119].

Portero et al. investigated the potential of chitosan glutamate in enhancing the absorption of ^{3}H-mannitol-labeled and fluorescein isothiocyanate-labeled dextrans with various molecular weights (4.4–19.5 kDa) across the buccal TR146 cell culture model. Chitosan glutamate concentrations of 20 μg/mL and higher were effective in enhancing the transport of these hydrophilic molecules across the TR146 cell culture model. The possible mechanism could be the disorganization of intercellular lipids in buccal epithelium[120].

Sandri et al. evaluated four chitosan derivatives as buccal and vaginal penetration enhancers through porcine cheek and vaginal mucosa. The four derivatives selected were 5-methyl-pyrrolidine chitosan (MPC), two low-molecular-weight chitosans (DC1 and DC2), and a partially reacetylated chitosan (RC). Chitosan HCl was used as a reference and acyclovir as a model drug. Among four derivatives, MPC showed the best buccoadhesive and penetration-enhancing properties in both buccal and vaginal environments[121]. Researchers also investigated the influence of quaternization of *N*-trimethyl chitosans (TMCs) on their mucoadhesive and penetration-enhancing properties toward buccal mucosa using a hydrophilic high-molecular-weight fluorescein isothiocyanate dextran (FD4; 4400 Da). TMC derived from the lower MW chitosan and characterized by the highest degree of quaternization showed the best mucoadhesive and penetration enhancement properties[122]. Sandri et al. studied the penetration enhancement properties of chitosan hydrochloride (both as a polymeric solution and as a nanoparticulate system) and of TMC hydrochloride on porcine buccal mucosa. TMC and chitosan nanoparticulate systems were able to increase FD4 permeation across buccal epithelium to a greater extent than the chitosan solution[123].

N-TMC chloride, a partially quaternized derivative of chitosan, has been reported as a polymeric absorption enhancer for improved peroral delivery of peptide drugs (buserelin and octreotide) after intraduodenal or jejunal administration. This methylated derivative is also able to increase the permeation of hydrophilic compounds such as ^{14}C-mannitol and ^{14}C-PEG across the cell monolayers. The proposed mechanism is opening of tight junctions located between epithelial cells, which results in permeation enhancement via the paracellular route[124].

Langoth et al. evaluated the permeation-enhancing effect of chitosan, chitosan–thioglycolic acid (TGA) conjugate (1%, m/v), reduced glutathione (GSH, 2%, m/v), and chitosan–TGA conjugate in combination with GSH on absorption of pituitary adenylate cyclase-activating polypeptide (PACAP) through porcine buccal mucosa using Ussing chambers. The permeability coefficient increased to 1.5-fold, 3.51-fold, 2.75-fold, and 10.1-fold with chitosan, chitosan–TGA conjugate, GSH, and chitosan–TGA conjugate in combination with GSH, respectively, compared to PACAP alone. The apparent permeability coefficient of PACAP in buffer was $(5.7 \pm 3.1) \times 10^{-8}$ cm/s while it was $(8.5 \pm 3.1) \times 10^{-8}$, $(15.69 \pm 3.06) \times 10^{-8}$, $(20.04 \pm 3.4) \times 10^{-8}$, and $(57.34 \pm 31.7) \times 10^{-8}$ cm/s in the presence of chitosan, GSH, chitosan–TGA conjugate, and chitosan–TGA conjugate in combination of GSH, respectively[125]. This showed that chitosan–TGA conjugates are better than chitosan alone and are promising carriers for buccal administration of PACAP.

Later, the authors also studied the effect of SDC, cetrimide (5% each), and chitosan-4-thiobutylamidine conjugate (chitosan–TBA, 1%) in combination with GSH (2%) on the permeation of PACAP using the same tissue. The enhancement ratio of 18.6-fold, 46.5-fold, and 77.5-fold was obtained with SDC, cetrimide, and chitosan–TBA in combination with GSH, respectively. It proves that chitosan–TBA conjugates are promising as buccal permeation enhancers for peptide drugs like PACAP[126]. They performed in vivo studies (in pigs) of thiolated chitosans as mucoadhesive and permeation-enhancing agents for the buccal delivery of PACAP. Formulations containing thiolated chitosans showed absolute bioavailability of 1% and a continuously raised plasma level of the peptide drug was maintained within the therapeutic range during the duration of study (6 hours), whereas PACAP did not reach the systemic circulation when administered with unbound chitosan[127].

From all the data available in literature, it appears that major mechanism of oral mucosal permeation enhancement by chitosan (and its derivatives) could be its bioadhesive nature, which increases the retention of the drug at mucosal surface. This could also minimize the drug loss by salivary flow[85].

Apart from chitosan, various grades of hyaluronic acid were also explored as penetration enhancers for buccal, vaginal, and intestinal delivery of therapeutic agents. Sandri et al. compared mucoadhesive and permeation-enhancing properties of three grades of hyaluronic acid (1878, 693, and 202 kDa) with chitosan HCl using porcine buccal and vaginal tissue, CaCo-2 cell lines, and rat jejunum. Acyclovir was the model drug. All grades of hyaluronic acid showed mucoadhesive properties in buccal, vaginal, and intestinal tissues. Hyaluronic acid with the lowest molecular weight (202 kDa) exhibited the best penetration enhancement properties. These effects were either comparable with or even better than chitosan HCl[128].

Cyclodextrins

Despite the increased bioavailability of hepatically metabolized drugs through buccal delivery, poor solubility of drugs in saliva may impede drug release from its device for uptake by buccal mucosa. Solubilization of a poorly water-soluble drug by complexing with

cyclodextrin and delivering via oral mucosa is advantageous in increasing drug absorption and bioavailability. Hydroxypropyl-β-cyclodextrin (HP-β-CD) has been reported as a stabilizing agent for ovine growth hormone, interleukin-2, and bovine insulin[129].

Pitha et al. reported that the buccal delivery of various steroidal hormones could be enhanced by the co-administration of condensation products of β-cyclodextrin with epichlorhydrin or propylene oxide. The inclusion complex formed showed better absorption of the drug in saliva[130]. Cyclodextrin complexation also showed enhanced sublingual bioavailability of testosterone[131] and clomipramine[132].

Imidazole antimycotics (e.g., miconazole, econazole, and clotrimazole) are extensively used in local treatment of fungal infections in the oral cavity. Because of their low water solubility and high lipophilicity, they were released extremely slowly from the lipophilic chewing gum base. Formulating HP-β-CD inclusion complex of these antimycotics into chewing gums was found to increase the drug release from them[133].

Jain et al. developed mucoadhesive buccal tablets of danazol sulfobutylether-7 (SBE 7) β-cyclodextrin complex using different polymers [polycarbophil and hydroxypropyl methyl cellulose (HPMC)]. These tablets were evaluated for their in vitro release, bioadhesion properties, and for drug absorption in female beagle dogs. In vivo bioavailability studies showed that perorally administered danazol–SBE 7 complex and the danazol–SBE 7 (in polycarbophil matrix) buccal tablets had absolute bioavailabilities of 64% and 25%, respectively, which were significantly greater than 1.8% observed for the commercial formulation, Danocrine. Increased bioavailability was attributed to the enhanced solubility because of complexation and the possible avoidance of extensive hepatic metabolism upon buccal administration[134].

Cappello and co-workers studied the feasibility of HP-β-CD in enhancing the in vitro permeability of a poorly water-soluble drug (carvedilol) through porcine buccal mucosa from poly(ethyleneoxide) buccal tablets. The amount of drug permeated from PEO tablet was higher in case of HP-β-CD-containing tablets, and there was a 20-fold increase in the amount of drug permeated through mucosa. The mechanism responsible for this could be increase in erosion rate of the tablet by HP-β-CD and improved dissolution of drug inside the polymeric matrix[135].

Miscellaneous

There is a wide range of structurally different compounds that can modulate the buccal permeation of drugs. Some of them are proven skin penetration enhancers, but their buccal permeation performance, irritation potential, and long-term toxicity is yet to be established. Their mechanism of action depends on their structure and may or may not be similar to the mechanism(s) cited above. Lecithin and its derivatives are potential candidates for drug delivery within the oral cavity. Nantwi et al. reported the presence of target receptors on human buccal cells and rat oral epithelium, which are responsible for binding of lecithin to the tissue. Binding is important for dosage form retention and can be affected by the time of exposure and the presence of saliva[136].

Coleman manufactured oral dissolvable dosage forms containing various permeation enhancers, which were capable of modifying the permeability of mucosal tissues of mouth, pharynx, and esophagus in order to facilitate the absorption of drugs through the oral epithelium[137].

Ethanol has been shown to enhance the permeability of tritiated water and albumin across ventral tongue mucosa and to enhance the permeability of CAF through porcine buccal mucosa via paracellular route[138,139]. The mechanism responsible for this could be the disruption of lipid molecules by ethanol, which affects intercellular domains of the epithelium.

Synergism in permeation enhancement was observed when ethanol was co-administered with fatty acids and/or their esters. Ethanol, oleic acid, and water (49:1:50) combination enhanced (13-fold enhancement) the ex vivo oral mucosal permeation of LH through pig mucosa[105]. Permeation of 17-β-E2 was effectively enhanced across golden hamster cheek pouch when ethanol (40%) and glyceryl monolaurate (2%) were used as permeation enhancers[140]. Permeation enhancement was also observed when ethanol/PG and sodium caprate were used in combination.

In vitro permeation of caffeic acid phenetyl ester was significantly enhanced from a topical mucoadhesive gel containing propolis using a combination of PG and Tween 20. Porcine buccal mucosa was used as model membrane. The combination of PG and Tween could be useful both as a solvent and as an enhancer. The increase in flux of propolis was attributed to the improvement in its solubility in the presence of enhancer[141].

Azone is a well-established skin permeation enhancer. Its enhancing effect has been attributed to a disruption of the organized lipid structure in the intercellular region of stratum corneum of skin, resulting in increased lipid fluidity and enhanced drug diffusivity. This mechanism may also be true when azone (AZ) is used as a buccal permeation enhancer. Kurosaki et al. reported that AZ increased the in vitro and in vivo buccal permeation of salicylic acid through hamster cheek pouch by increasing the lipid fluidity[142]. Hamster cheek pouch mucosa has keratinized surface that closely resembles the stratum corneum of skin. That could be the reason that Azone showed similar mechanism in

both the membranes. The results obtained using keratinized buccal membranes may not be directly correlated with the results to be obtained from nonkeratinized membranes as both membranes have different nature and organization. Whether AZ would have the same enhancing effect through nonkeratinized buccal mucosa is yet to be established.

Nicolazzo et al. assessed the buccal mucosal uptake and retention of triamcinolone acetonide (TA) in the presence of AZ using porcine buccal mucosa. Pretreatment of the buccal mucosa with 5% AZ resulted in 4.4-fold increase in tissue concentration of the drug. The amount of drug in the tissue was increased from 3.40% of the initial dose in untreated tissue to 14.85% of the initial dose in AZ pretreated tissue. Mucosal uptake and permeability of TA was also determined using a marketed product, Kenalog in Orabase in the presence and absence of AZ. Incorporation of AZ in Kenalog in Orabase did not result in an enhanced tissue concentration of TA but when the tissue was pretreated with azone, significantly higher amounts of TA accumulated in the tissue. With pretreatment, it was expected that most of AZ would have partitioned into the superficial layers of buccal mucosa but when AZ was present in formulation, the amount of AZ that would have been released from the formulation and partitioned into the tissue would have been relatively lower[143].

Xu and co-workers investigated the hypoglycemic effect of insulin buccal spray in diabetic rats and rabbits containing a combination of soybean lecithin (SL) and propanediol as absorption enhancer. Results revealed that enhancers promoted the passage of insulin across the buccal epithelium by both intracellular and paracellular routes. The synergistic permeation-enhancing effect of these two compounds may be because of the hydrophilic property of propanediol, which lowers the hydrophobic property of SL[144].

Yang et al. explored the possibility of phospholipid-deformable vesicles for buccal delivery of protein drugs. Two kinds of vesicles, with and without the presence of sodium deoxycholate (deformable vesicles and conventional vesicles), were prepared using insulin as a model protein. The hypoglycemic effects, insulin concentrations, and residual amounts of insulin deposited in the buccal membrane after buccal administration of insulin vesicles to rabbits were investigated. The relative pharmacological bioavailability and the relative bioavailability of insulin-deformable vesicles were 15.59% and 19.78%, respectively, compared to subcutaneous administration of insulin solution. These values were significantly higher than the conventional insulin vesicles[145].

Cui et al. evaluated the effect of various enhancers on sublingual delivery of insulin. The effects on the sublingual mucosal lipid fluidity and aggregation states of insulin were estimated by fluorescence polarization and circular dichroism, respectively. HP-β-CD, egg lecithin, chitosan, oleic acid, Tween 80, POE lauryl ether, and polyethylne–poly-PG were used as enhancers, and human immortalized oral epithelial monolayer was used for evaluating the transport. These enhancers (except Tween 80) markedly reduced the fluidity of sublingual mucosal lipids and also had an influence on the secondary structure of proteins. The hexamers of insulin were dissociated to monomers only by chitosan, egg lecithin, and POE lauryl ether. The mechanism of permeation enhancement could be loosening the tight junction of epithelia, increasing the lipid fluidity of mucosa, and /or dissociating the hexamers of insulin to monomers, but the former one was the most likely (according to authors). Plasma glucose levels in rats were significantly lowered after administration of insulin with an enhancer compared with those without an enhancer[146].

Zhu et al. investigated the permeation-enhancing effect of SL-based vesicles on penetration of insulin through porcine buccal mucosa using Valia-Chien diffusion cells. The permeation of insulin from SL-based vesicles was three times higher from that of control solution[147].

Starokadomskyy and Dubey studied the permeability of α-interferon and FITC-insulin through hamster cheek pouch buccal mucosa in the presence of lysalbinic acid (LA). LA, a product of alkaline hydrolysis of egg albumin, has mild detergent properties. It was shown that LA significantly enhanced the permeation of α-interferon and FITC-insulin via the paracellular route. The molecular mechanism responsible for this is not clear yet but it may be similar to that of other surfactant enhancers (SDS and other bile salts) based on intercellular lipid solubilization[148].

Eichman and Robinson demonstrated that effervescence could be a potential permeation enhancer for molecules through biological membranes. These researchers performed a series of experiments by bubbling large volumes of CO_2 through the donor compartment of modified Ussing diffusion chambers and found enhanced permeation of drugs through rabbit ileum epithelium. The possible mechanism responsible for enhancement could be a solvent drag effect because of increased blood flow, opening of tight junctions, and/or increasing the hydrophobic nature of the cell membrane[149].

Scientists explored the potential of effervescence for permeation enhancement through oral mucosal membranes[150]. Durfee and his co-workers developed a platform technology using effervescent agent(s) and pH modifier(s) to enhance buccal delivery of fentanyl, a poorly soluble weak base useful for the relief of cancer pain. Enhanced absorption of drug was achieved

primarily by dynamic shifts in pH within the microenvironment lying between a dissolving tablet and buccal mucosa. Initially, dissolution of citric acid causes initial drop in pH, which combines with bicarbonate to form carbonic acid. Carbonic acid dissociates into CO_2 and water. As CO_2 releases, the pH increases, which favors the formation of nonionized fentanyl. The nonionized form of drug readily permeates through the buccal mucosa[151]. Clinical studies in healthy human volunteers revealed faster and complete absorption and higher bioavailability of fentanyl from effervescent buccal tablets compared with noneffervescent buccal tablets[152].

Patents/patent applications on permeation enhancers used for oral mucosal delivery of therapeutic agents (2001–2009) are tabulated in Table 4, and permeation enhancers studied for the oral mucosal delivery of therapeutic agents (1998–2009) are summarized in Table 5.

Prodrugs and enzyme inhibitors

Prodrug approach has been extensively used to protect drugs against degradation during penetration. But use of this approach to overcome the metabolic barrier of buccal mucosa against various pharmacologically active agents has not been fully established. Among mucosal routes, the buccal mucosa has relatively low enzymatic activity and drug inactivation is neither rapid nor extensive[153]. However, enzymes in saliva and buccal mucosa can still degrade drugs, particularly peptides/proteins as several proteolytic enzymes are found in buccal epithelium (Table 2). Although buccal mucosa appears to have low basal cytochrome P-450 (CYP) activity[57,154], other non-CYP metabolic processes in this tissue have also been reported. Among them, different types of peptidases and esterases have been identified in either human or animal buccal specimens[55,155]. In addition, buccal metabolism of drugs that are subjected to peptidase-mediated and/or esterase-mediated degradation has been found to have substantial effect on their delivery through the buccal route[16,55]. Therefore, buccal administration of compounds that may be liable to these enzymatic processes may present a challenge.

Co-administration of a drug with enzyme inhibitors is another strategy for improving the buccal absorption

Table 4. Patents/patent applications on permeation enhancers used for oral mucosal delivery of therapeutic agents (2001–2009).

S. no.	Patent no.	Drug candidate(s)	Absorption enhancer(s)	Dosage form(s)	Ref.
1	EP 1146890	Ozone inactivated toxin	Benzalkonium chloride	Aerosol spray	Mundschenk[200]
2	US 6375975	Insulin	SDS + lecithin, sodium hyaluronate, glycine chenodeoxycholate, etc.	Spray	Modi[201]
3	US 2003171259	Insulin	SDS + lecithin + hyaluronic acid	Spray	Modi[202]
4	US 2003059376	PTH, growth hormone, insulin	SDS, chlorbutanol, cyclodextrin, etc.	Spray	Libbey et al.[203]
5	US 6676959	Nicotine	Egg lecithin and/or soyalecithin	Tablets and other solid dosages	Andersson et al.[204]
6	WO 2004016243	Insulin, fentanyl	SDS + micelle forming agents s.a. lecithin, sodium hyaluronate, chenodeoxycholate, etc.	Spray	Modi[205]
7	GB 2394415	Selegiline	Lauroyl macroglycerides and PEG 600	Disintegrating tablet	Moonga[206]
8	US 6849263	Morphine, fentanyl	SDS + polyoxyethylene 9 lauryl ether + SGC	Spray	Modi[207]
9	WO 2005115339	Various agents	L-α-lysophosphatidylcholine	Solid dosage forms	Mathias and Li[208]
10	US 6974590	Fentanyl citrate, prochlorperazine	Citric acid and sodium bicarbonate	Tablet	Pather et al.[150]
11	US 2006002989	Sumatriptan succinate	Sodium caprate	Tablet	Ahmed et al.[209]
12	WO 2006010939	Insulin	• SDC and lecithin • Dextran sulphate and/or dimethyl and sulfobutyl cyclodextrins • EDTA and SDC	Film or wafer	Martyn and Coghlan[210]
13	US 2007014735	Peptides (oxytocin, arginine, etc.)	Quaternary ammonium salts	Spray	Mundschenk[211]
14	US 20070207192	Acyclovir, sumatriptan, naratriptan	• Capsaicin and/or tocopheryl acetate	Gel/patch	Holl and Osborne[212]
15	WO 2008137054	Polypeptides (calcitonin, glucagons, etc.)	• STC	Tablet	Durfee and Thurman[213]
16	US 2009047328	Caffeine	• Taurine • Soya lecithin	Strip/gel	Cunningham[214]

Table 5. Permeation enhancers studied for the oral mucosal delivery of therapeutic agents (1998–2009).

S. No.	Drug(s)	Enhancer(s)	Model	Results	Ref.
In vitro/ex vivo methods					
1.	Morphine HCl	SGC (10 and 100 mM)	Porcine	Enhancement factor of 2 at 100 mM. No enhancement at 10 mM	Senel et al.[215]
2.	Basic fibroblast growth factor	SGC (15 mM)	Rabbit	Flux ↑ from 1.4 ± 0.13 ng/min/cm to 3.2 ± 0.13 ng/min/cm	Johnston et al.[216]
3.	Lidocaine HCl	• Sucrose fatty acid esters	Porcine	22-fold ↑ in the enhancement ratio with sucrose laurate	Ganem-Quintanar et al.[105]
		• Ethanol + oleic acid + water		13-fold ↑ in the enhancement ratio with ethanol, oleic acid, and water	
4.	Acyclovir	SGC	Porcine	Significant ↑ in the permeation of the drug in the presence of enhancer	Shojaei[81]
5.	Hydrocortisone	Chitosan H (2%)	Porcine	Significant ↑ in flux. Sixfold increase in permeation	Kremer et al.[118]
6.	Dideoxycytidine	Menthol	Porcine	Significant ↑ in permeation in the presence of menthol	Shojaei et al.[217]
7.	Mannitol (hydrophilic marker)	STC, SGC, STGC	Porcine, TR146 cells	STC increases the apparent permeability of mannitol through both models	Nielsen and Rassing[54]
8.	DADLE	Glycerol monooleate	Porcine	Significant ↑ in flux by the enhancer	Lee and Kellaway[107]
9.	Sumatriptan	SGDC and lauric acid (1:1)	Human	No enhancement in permeability with enhancers	Van der Bijl et al.[218]
10.	ISIS 3082	SGC (100 mM)	Porcine	Permeation of the drug ↑ in the presence of enhancer	Jasti et al.[219]
11.	Triamcinolone acetonide	Surfactants, glycols, SDC	Porcine	SDC showed the best enhancing effects	Chin and Kim[220]
12.	Nitrosonornicotine	Ethanol + nicotine	Perfusion system	Synergistic effect in the presence of ethanol + nicotine	Du et al.[221]
13.	Carbamazepine	SGDC	—	No significant difference with/without SGDC	Ikinci et al.[222]
14.	Transforming growth factor-β	Chitosan-H (2%)	Porcine	Significant ↑ in flux of the drug across the buccal mucosa	Senel et al.[119]
15.	Endomorphin	SGC, STC	Porcine	SGC, STC were ineffective in enhancing permeation	Bird et al.[166]
16.	Ergotamine tartarate	CLOE (5%)	Guinea pig	Permeation of the drug ↑ significantly using CLOE	Tsutsumi et al.[103]
17.	^{3}H-mannitol and FITC-dextran	Chitosan glutamate (1, 20, 40, 60, and 100 μg/mL)	TR146 cell culture	Permeability coefficients ↑ for both hydrophilic agents across the cell culture model	Portero et al.[120]
18.	Triamcinolone acetonide	Triethyl citrate, castor oil	—	Slow release rate of the drug from the polymeric films with enhancers	Chun et al.[223]
19.	Propranolol	LPC, DDPC, STDHF (0.5%), ethanol (20% and 30%)	—	LPC, DDPC, STDHF, and ethanol showed enhancement ratios of 3.2, 2.9, 1.0, and 1.5, respectively	Lee and Choi[224]
20.	Thiocolchicoside	STC, STDC	Porcine	No increase the flux of the drug	Artusi et al.[225]
21.	Diclofenac sodium	SDC, STC, STDC, menthol	Chicken	No remarkable effect of the enhancers on the flux of the drug	El-Samaligy et al.[226]
22.	Buspirone	PG (40%) + oleic acid (5%)	Porcine	↑ flux in the presence of enhancer	Birudaraj et al.[100]
23.	Acyclovir	DC1, DC2, RC MPC	Porcine	MPC showed the best permeation enhancement	Sandri et al.[121]
24.	Acyclovir	Hyaluronic acid-HA (1878, 693, and 202 kD)	Porcine	202 kD HA exhibited the best penetration enhancement properties	Sandri et al.[128]
25.	Triamcinolone acetonide	AZ (5%)	Porcine	4.4-fold ↑ in tissue concentration of the drug	Nicolazzo et al.[143]
26.	Insulin	Soybean lecithin	Porcine	Three times higher permeation of insulin from SL-based vesicles	Zhu et al.[147]

(*Continued*)

Table 5. (*Continued*).

S. No.	Drug(s)	Enhancer(s)	Model	Results	Ref.
27.	Lorazepam	CPC, polyethoxylated castor oil (PCO) and PEG dodecyl ether	Cultured buccal epithelium (human); Hamster	Presence of enhancers increased the drug permeation rate (except when present at the higher concentrations)	Burgalassi et al.[109]
28.	Nimesulide	Ethanol (20%, v/v), isopropyl myristate, STC (20 mM)	Porcine	Permeation of both ionized and unionized species was increased after pretreatment with enhancers	Goswami et al.[227]
29.	Naltrexone	Sodium dehydrocholate (0.1%), trisodium citrate trihydrate (0.5%), NaEDTA (1%)	Cultured cell layers Porcine	No significant effect of the enhancers on the permeation rate of the drug through the membrane using both artificial and natural human saliva	Giannola et al.[171,172]
30.	Sumatriptan succinate	Dimethyl sulfoxide (3%)	—	Increase in permeation of drug in the presence of enhancer	Shidhaye et al.[228]
31.	Atenolol (A), metoprolol (M), propranolol (P)	SGDC (0.5–5 mM)	Porcine; HO-1-u-1 model	*Porcine sublingual mucosa*: 30-fold ↑ in permeability of A (1–2.5 mM SGDC); significant ↑ for M (2.5 mM SGDC); no ↑ for P. *HO-1-u-1 cell culture model*: Significant ↑ for A (2.5–5 mM SGDC); no ↑ for P	Wang et al.[229]
32.	Diclofenac-free acid (DicH) and Na salt (DicNa)	HP-β-CD	Porcine; silicon membrane	↑ flux of DicH in the presence of HP-β-CD. Decrease in flux of DicNa in the presence of HP-β-CD.	Miro et al.[230]
33.	Omeprazole	β-cyclodextrin (β-CD) and M-β-CD	Porcine	↑ drug permeation in complexed form of 1.1- and 1.7-fold for β-CD and M-β-CD, respectively	Figueiras et al.[231]
34.	Quinine HCl	HP-β-CD	Porcine	Highest drug flux from the inclusion complex of drug:HP-β-CD in 1:1 molar ratio	Ong and Heard[232]
35.	5-Fluorouracil (FU)	SDC, SDS, STGC, oleic acid	Rabbit	STGC was found most effective than other enhancers. Order was STGC > SDS > SDC > oleic acid	Dhiman et al.[233]
In vivo methods					
1.	Leuprolide	Benzoic acid, ethanol, peppermint oil	Dogs, monkeys, humans	*Abs. bioavailability*: 46.7% in dogs, 2.7% in monkeys (dose 0.45 mg/kg), 2.4% in humans (dose 4.5 mg)	Qiu et al.[234]
2.	Triamcinolone acetonide	SDC	Rabbit	There was 1.59-fold ↑ in F_R in the presence of enhancer	Chin et al.[235]
3.	Insulin	Soybean lecithin + propanediol	Rats, rabbits	Enhancers promoted the passage of insulin across the buccal epithelium	Xu et al.[144]
4.	Thiocolchicoside	STC, STDC	Humans	No increase the flux of the drug	Artusi et al.[225]
5.	Denbufylline	SGDC	Rabbit	SGDC aids the penetration of epithelial permeability barrier and improves the transport of the drug	Martin et al.[236]
6.	Insulin	SS, SDC, Witepsol (3%), polyoxyethylene 9 lauryl ether (1%)	Porcine	*Without enhancer*: 6.72% relative hypoglycemia, RH; POELE, 9.42% RH; SS, 7.93% RH; SDC, 8.65% RH	Honsy et al.[237]
7.	Piroxicam	SDS (1%), SDC (3%), STGC (3%)	Humans	The gel formulations were better than or equally effective to the orally administered commercial product	Attia et al.[238]
8.	Lorazepam	CPC, polyethoxylated castor oil (PCO), PEG dodecyl ether	Rabbits	Only CPC at the highest tested concentration was moderately active	Burgalassi et al.[109]
9.	5-Fluorouracil (FU)	STGC	Rabbits	Absolute bioavailability of FU from mucoadhesive gels containing STGC increased 1.6-fold as compared to gels without permeation enhancer	Dhiman et al.[233]

of drugs, particularly proteins and peptides. Protease inhibitors such as aprotinin, bestatin, puromycin, and some bile salts stabilize protein drugs by affecting activity of proteolytic enzymes, altering the conformation of the peptides or proteins, forming micelles, and/or rendering the drug less accessible to enzymatic degradation[16,153].

Yamamoto et al. found that the metabolism of insulin and proinsulin has been reduced by aprotinin (a serine protease inhibitor) in homogenates of rabbit buccal mucosa[156]. Similarly, bestatin and puromycin showed reduced enzymatic activity when incorporated with 4-methoxy-2-naphthylamides of leucine, arginine, alanine, and glutamic acid[157].

Garren et al. performed absorption test with D- and L-isomers of leucine *p*-nitroanilide (LPNA) in an isolated hamster cheek pouch. Only L-isomer was sensitive to enzymatic degradation in the buccal mucosa. In diffusion experiments with L-LPNA, no drug transport could be measured, indicating that enzymatic activity overrules the amount of drug that has crossed the mucosa. Bestatin, an aminopeptidase inhibitor, was able to increase drug transport, whereas D-LPNA showed linear diffusion characteristics. By splitting the epithelium from the underlying tissue with collagenase, they were able to locate the enzymatic activity in epithelial layer[28].

Absorption enhancers like bile salts can also be used to overcome the metabolic barrier of buccal mucosa. Nakada et al. found that the degradation of calcitonin was reduced to about 55% by SDC and to about 67% by STC when incubated in a homogenate of rat mucosal tissue at pH 7.4. However, when it was incubated alone, the degradation was 90% after 20 minutes[158].

Hussain et al. reported an increase in bioavailability of opioid analgesics and antagonists (nalbuphine, naltrexone, naloxone, oxymorphone, butorphanol, and levallorphan) by esterification of their 3-phenolic hydroxyl groups. In this way the bitter taste, the main threshold for buccal delivery and the cause for overproduction of saliva, was avoided. While orally administered analgesics were absorbed to an extent of 5%, the buccal delivery resulted in a bioavailability of 90% in rats and 35–50% in dogs without visible adverse effects. Plasma peak levels were established after 1 hour[159].

Hansen et al. improved the flux of ketobemidone across porcine buccal mucosa in vitro by converting its phenolic hydroxyl group to a carboxylic acid or a carbonate ester. The prodrug was found to be more lipophilic and resistant to saliva-catalyzed hydrolysis[160].

Bundgaard and Moss have developed a series of bioreversible derivatives (*N*-alkoxycarbonyl derivatives) of TRH to improve its lipophilicity and resistance to cleavage by TRH-specific serum enzymes[161]. Similarly, Bundgaard and Rasmussen have formulated various prodrugs (*N*-benzyloxycarbonyl derivatives and *N*-hydroxymethyl derivatives) of peptides to overcome the metabolic barrier imposed by carboxypeptidases and aminopeptidases. They found that number of these derivatives were resistant to proteolytic cleavage at pH 7.4 and 37°C. It is evident that derivatization will result in improved buccal bioavailability of peptide drugs by reducing their metabolism[162].

Christrup et al. developed ester prodrugs of morphine to improve its buccal delivery. In vitro permeability studies showed that morphine-3-propionate (M3P) had better permeability than morphine-3-acetate (M3A) through porcine buccal mucosa. In contrast, M3A showed better in vivo absorption than M3P. This could be explained by enzymatic stability of two esters in saliva. M3P was more rapidly hydrolyzed in saliva than M3A. The study demonstrated that buccal delivery of morphine can be markedly improved by using ester prodrugs with higher lipophilicity than the parent drug[163].

Veuillez et al. studied the ex vivo permeation of a model peptide, tryptophan–leucine (Trp–Leu) in two different regions of pig oral mucosa, the hard palate and the cheek. They synthesized a lipophilic derivative of the peptide by acylation of the N-terminal amino group of Trp–Leu with myristic acid to increase its mucosal absorption. High-performance thin-layer chromatography studies showed that the purified Trp–Leu derivative (Myr–Trp–Leu) was more lipophilic than parent Trp–Leu. The parent peptide was unable to pass through the keratinized layer of palatal mucosa, and after 24 hours only 12% had passed through buccal mucosa to the receptor compartment, whereas 25% and 70% of the acylated peptide was found in the palatal and buccal mucosae, respectively[164]. This acylated derivative also showed higher partition coefficient in *n*-octanol/phosphate buffer pH 7.4 (1.04 in comparison to −0.68 for parent peptide).

Walker et al. investigated the peptidase activity on the surface of porcine buccal mucosa. Aminopeptidases appeared to represent a major metabolic barrier to the buccal delivery of peptide drugs. Absence of endopeptidase and carboxypeptidase is advantageous for buccal delivery of peptide drugs[165]. Esterase activity is important with respect to the buccal delivery of ester drugs or prodrugs[55].

Bird et al. investigated the feasibility of buccal delivery of a model peptide, endomorphin-1 (EN-1). Diprotin-A, a potent inhibitor of dipeptidyl peptidase IV, provided significant inhibition of the degradation of EN-1 in buccal epithelium. In vitro permeation studies revealed that the permeability coefficient of EN-1 across porcine buccal epithelium was $5.67 \pm 4.74 \times 10^{-7}$ cm/s. Enzymatic degradation of the peptide was found not to be rate limiting to the drug's permeation across buccal epithelium, as diprotin-A did not increase the permeation of EN-1[166].

Maffei et al. performed a study to improve the flux of nimesulide across the buccal mucosa by using the drug as sodium salt. The salt was formulated as buccoadhesive tablets. The in vitro release studies were carried out in modified Franz diffusion cell through porcine buccal mucosa. The advantages of a higher concentration gradient for the flux related to higher solubility of the salt and to a sufficiently high permeation coefficient of drug despite the ionized form could not be completely exploited, because composition of the formulation destroys chemical form of the drug[167].

Physical methods (mechanical penetration enhancers)

Drug absorption can also be enhanced mechanically, by removing outermost layers from epithelium to decrease the barrier thickness, or electrically, by application of electric fields or by sonophoresis. The latter acts by reducing, temporarily, the density of lipids in the intercellular domain of the membrane. This disruption occurs because of a combination of micromechanical, thermic, and cavitation effects that effectively 'open up' the intracellular pathways, allowing substances to penetrate[168].

After chemical enhancement, the most efficient permeation enhancement methods for intraoral applications are electrical mechanisms, such as electrophoresis (iontophoresis), electro-osmosis, and electroporation.

Iontophoresis

The phenomenon of iontophoresis is known since 1900[167] and typical area of its application in pharmaceuticals is transdermal drug delivery. It may also be used for enhancement of drug transfer through buccal mucosa. Electrophoretic enhancement in the oral cavity has been reported for a number of applications[170–173]. It is most effective for water-soluble, ionized compounds. Currents below 0.5 mA/cm^2 are reported to be applied without adverse effects[174]. The rate of migration is limited by the maximum electric current, which can be applied across the mucosa. The current density limitation may be more restrictive than for skin, because of the bigger sensitivity to pain; however, it is expected that buccal administration via iontophoresis is more efficient and will require lower current density than transdermal application[175]. The buccal mucosa, being an absorptive mucosa, enables design of electronic intraoral implants for long-term-controlled delivery[168] [view Record in Scopus, cited in Scopus (1)] and invention of devices for needleless drug 'injection'.

Electro-osmosis

Another means of increasing the drug transport rate is by utilizing electro-osmosis. Human tissue possesses fixed negative charge and binds mobile, positive, counterions, forming an electrically charged double layer in the tissue capillaries. When an electric field is applied across the tissue, there is a net flow of water through the tissue through migration of the mobile solvated counterion, a process known as electro-osmosis. Drugs dissolved in interstitial water are transported into the tissue by bulk flow.

Electroporation

In *electroporation*, very short (10 μs–1 ms) high-potential (20–100 V) pulses are applied across the tissue. Because of electrostriction forces, cellular membranes are temporarily perforated or even microchannels in the tissue are formed. Those channels serve as a drug transport route and are closed within few minutes without any lasting damage to the tissue[85,122,176] [view record in Scopus, cited in Scopus (15)].

Mechanism of action of oral mucosal permeation enhancers

There is a lot of similarity between oral mucosal membrane and epidermis of skin. Both are stratified epithelial membranes, and the spaces between multiple layers of flattened cells are filled with lipids and glycolipids that were extruded from the membrane coating granules of the cells[177]. The oral mucosal membranes are generally more permeable than skin. This is because the former are considered to be less keratinized and intercellular lipids are loosely packed and therefore less structured than later[178]. This suggests that the mechanism, which is related to skin permeation enhancement, may or may not hold true for oral mucosal membranes. Fatty acids, when used as skin permeation enhancers, have been shown to increase the fluidity of intercellular lipids of stratum corneum. These fluidity changes increase the permeabilities of salicylic acid and piroxicam through skin. Azone also followed the similar mechanism. It has been well established that many skin penetration enhancers increase the diffusivity of permeants by disturbing the ordered intercellular lipid lamellae. This may not hold true in case of buccal mucosa, as these lipids are already in a less-organized state[178]. Theoretically, an improvement in permeability would be expected if agents could reduce the viscosity of intercellular matrix of buccal mucosa. However, there is no such evidence in the literature, so it seems that permeation enhancers exert their effects through alternative mechanisms[85]. The mechanism of action and limitation(s) of various classes of permeation enhancers are summarized in Table 6. Following are the mechanisms by which penetration enhancers are thought to improve mucosal absorption.

Table 6. Mechanism of action and limitation(s) of various classes of permeation enhancers.

Class	Mechanism(s)	Limitation(s)
Surfactants	√ Membrane interaction; enzyme interaction	× Damage and irritation to the mucosal membrane
	√ Extraction of membrane proteins and intercellular lipids	× Swelling of buccal mucosa
	√ Solubilization of peptides and other components	× Sloughing of epithelial surface
Bile salts	√ Denaturation of proteins	× Damage and irritation to the mucosal membrane
	√ Extraction of membrane proteins and intercellular lipids	× Swelling inside the cells, loss of upper cell layers, vacuole formation
	√ Decrease in mucus viscosity; decrease in peptidase activity	× Sometimes absorption of drugs decrease instead of increasing due to micellar solubilization
	√ Solubilization of peptides	
	√ Formation of reversed micelles	
Fatty acids	√ Increase fluidity of phospholipid domains by causing disruption of phospholipid acyl chain	× Unsaturated fatty acids may cause epithelial cell damage
	√ Influence on paracellular and transcellular routes	× Cytotoxic at higher concentration
Chelators	√ Ca^{2+} complexation thereby opening tight junctions and influencing paracellular permeability	× Interfere with the absorption of other nutrients
Cyclodextrins	√ Inclusion of membrane compounds	—
Positively charged polymers (chitosan and derivatives)	√ Ionic interaction with the negative charge on the mucosal surface	—
	√ Combination of bioadhesion and reversible interaction with components of tight junctions, leading to widening of paracellular route	
	√ Fluidization of organized intercellular lipid lamellae	
Azone	√ Decrease packing order of lipids by increasing the fluidity of intercellular lipids	× Irritation and toxicity to the membrane
Ethanol	√ Lipid extraction	× Severe histological and pathological changes on long-term use
	√ Denaturation or change in conformation of proteinaceous material	
	√ Co-transport mechanism	× Increase absorption of carcinogens when used along with tobacco
	√ Changing the partition coefficient of the solute by altering its solubility in the solute	

Altering mucus structure and rheology

Mucus is a highly viscous liquid adhering to the mucosa. It is secreted by goblet cells. The mean thickness of this layer varies from 50 to 450 μm in humans[179]. It is covered by a layer of saliva and forms a viscoelastic layer of varying thickness that affects drug absorption. There are evidences in the literature that saliva can hinder absorption of certain drugs but usually it is insignificant compared to other barriers during passage through buccal mucosa[16,22,26]. Therefore, saliva may have only a transient contribution to the oral mucosal barrier function. Bile salts decrease the viscosity and hence the rheological properties of the mucus to aid absorption.

Increasing fluidity of cell membrane

This is the most common mechanism of permeation enhancement for fatty acids and azone. These enhancers act by disturbing the intracellular lipid packing by interaction with either lipid or protein components. Azone and dimethylamino acetate have been shown to decrease the packing order of lipids by increasing the fluidity of intercellular lipids of buccal epithelial cells[180].

Extraction of intracellular lipids or denaturation of cellular proteins

This mechanism is mainly followed by surfactants and bile salts. DSC studies showed that sodium deoxycholate (1%) appeared to denature and extract proteins from rabbit buccal mucosa and also affect membrane lipids[30].

Affecting the components involved in the formation of intracellular junctions

Chelating agents affect the components involved in the formation of intracellular junctions. These agents chelate

with calcium ions present in tight junctions and increase the membrane permeability. As the intercellular junctions are virtually absent in oral mucosal membranes, this could be particularly important in case of intestinal membranes, where these junctions act as barriers to the passive diffusion of molecules. Enhancers that act mainly on epithelial junctions would be expected to have minor effect on the permeability characteristics of oral mucosa. About 47% of the intercellular space of the palatal stratum corneum is occupied by desmosomes (which are thought to provide added structural integrity) and further 10% is occupied by saccules. So, disruption of these structures may open a permeability pathway through the oral stratum corneum, but this has not been entirely established[181].

Overcoming the enzymatic barrier/enzyme inhibition

Various peptidase and proteases are present within the oral mucosa, and it is possible that metabolism could contribute to incomplete bioavailability. The inhibitors of these enzymes can act as potential penetration enhancers. However, changes in the membrane fluidity induced by the penetration enhancers may indirectly alter enzymatic activity.

Increasing the thermodynamic activity of drugs

This may be affected by the vehicle composition, which will influence drug solubility, and also by ion pair formation between the enhancer and the drug[182].

Disruption of lipid structure (formation of micelles)

This is also one of the important mechanisms of bile salts. Solubilization by formation of micelles (to create aqueous channels) results in permeation enhancement of hydrophilic molecules. These micelles also act as stabilizers for enzyme-liable drugs.

Toxicity and membrane damaging potential of permeation enhancers

Major disadvantage with the use of permeation enhancers, not only for oral mucosal drug delivery but also for all routes for drug delivery, is toxicity and membrane damage caused by them. Co-administration of permeation enhancers can potentially cause damage to mucosa of oral cavity[177]. There are numerous reports in the literature indicating that there is correlation between permeation enhancement and membrane damage. Several bile salts, ionic surfactants, and a fatty acid were investigated for their permeation enhancement properties of various compounds through hamster intestine and their damaging effects (histological and biochemical evaluation) on the same membrane (in vitro). The results showed that there is a direct relationship between membrane permeation enhancement and membrane damage[183]. The same relationship was also seen with AZ and its derivatives when used as skin penetration enhancers[184] and laureth-9, lysophosphatidylcholine, and a steroidal detergent when given intranasally to rats[185].

Niccolazzo and co-workers investigated the effect of various lipophilic skin penetration enhancers octisalate (OS), padimate O (PO), and AZ on in vitro buccal permeability of CAF, E2, and TA through porcine buccal mucosa. E2 buccal transport was not altered following octisalate pretreatment but was reduced by 26.3% with PO pretreatment and 67.6% with AZ pretreatment. There was 4.1-fold increase in enhancement ratio of TA when treated with AZ. This enhancing effect was attributed to increase partitioning of drug into the tissue. Reduction in flux caused by PO and AZ may have been because of enhanced tissue retention of E2[139]. This showed that enhancers also have negative effects on the permeation rate of certain agents and are drug specific.

The toxicity potential of permeation enhancers depends upon their concentration and time of exposure. Boulmedarat et al. studied the toxicity of randomly methylated beta-cyclodextrin (RAMEB) on buccal mucosa using a reconstituted human oral epithelium model composed of TR 146 cells. Toxicity of RAMEB on TR 146 cells was evaluated by measuring cell viability (MTT assay) and membrane damages followed by LDH release after single and repeated exposures to RAMEB solutions. The findings indicated that 10% RAMEB resulted in cytotoxic and inflammatory effects depending on time exposure, whereas 2% and 5% RAMEB did not induce tissue damages even after 5 days of repeated exposures[186].

It has been shown that ionic surfactants (sodium lauryl sulfate and cetyl pyridiniumchloride) can damage and separate the keratinized layers of epithelium, with loss of surface squames when applied to ventral surface of tongue of dogs[187]. Sodium deoxycholate (0.5%) or sodium lauryl sulfate (0.1%) when applied to rabbit buccal mucosa showed loss of surface epithelial layers[30].

Nakane et al. investigated the mucosal irritation of a transmucosal therapeutic system formulated for the buccal delivery of LHRH, containing various bile salts (SC, SDC, and STDC) as permeation enhancers[188]. The irritation effects included erythema, edema formation, and eschar formation. These effects could be viewed with the naked eye. Senel et al. studied the mucosal irritation of various bile salts on buccal mucosa of porcine, bovine, and humans. The histological changes were loss of upper cell layers, decrease in number of desmosomes, separation of epithelium from connective

tissues, swelling inside the cells, and vacuole formation. These observations were determined by light and transmission electron microscopy[77,189].

There are some evidences suggesting that the effect of enhancers on membrane permeability have been reversible. Azone showed rapidly reversible effects on membrane permeability of hamster cheek pouch[142]. STC and lysophosphatidylcholine also exhibited similar reversible effects on membrane permeability of dog oral mucosa[190].

A transient increase in the fluidity of the intracellular lipids may be thought of as a relatively nontoxic effect, whereas extraction of the intracellular lipids or denaturation of cellular proteins may be viewed as being somewhat more drastic[80]. Therefore, it is very important to ensure that the effect of enhancer on membrane permeability is not permanent and membrane should revert to its normal integrity and barrier function upon removal of the enhancer[31,177].

Addition of secondary substances can be used to modulate the interaction between enhancers and mucosal membranes, in this way reducing irritative effects. It has been reported that the addition of lecithin or oleic acid and glyceryl monostearate protects the intestinal membrane against toxic effects of bile salts[28,30,191].

Conclusion

Oral mucosa can be an effective barrier to the absorption of several therapeutic agents, particularly macromolecular drugs, and co-administration of a permeation enhancer may be necessary to improve drug absorption through the mucosa. The potential membrane damage caused by the enhancers to the mucosal membrane is an important concern. Although most penetration enhancers were originally designed for purposes other than absorption enhancement, a systemic search for safe and effective penetration enhancers should be a priority in drug delivery.

Many permeation enhancers have been investigated both in vitro and in vivo and some are found to be very effective. The studies showed that, besides its permeabilizing effect, its favorable biological properties such as nontoxicity, biocompatibility, and biodegradability makes chitosan a promising candidate for a safe mucosal penetration enhancer[77]. But despite qualitative and quantitative research in this field, there is only one formulation for oral mucosal administration with penetration enhancer(s) that is available commercially, till date. It has been developed lately by Generex Biotechnology Corporation (Toronto, ON, Canada) and is a liquid formulation (*Oral-lyn*™) containing human recombinant insulin, combination of absorption enhancers (surfactants), and the *RapidMist*™ device. Generex receives regulatory approval to sell this product in India, Lebanon, and Algeria. The sizeable lag time between the lab scale research and commercial viability of products containing permeation enhancers can be attributed to the lack of satisfactory profile with respect to efficacy, irritation, and toxicity and lack of IVIV correlation data. Practically, it is not feasible to increase membrane permeability without compromising with its structural integrity. Although irritation caused by enhancers is not permanent and is reversible, a thorough understanding of the drug transport mechanisms is perquisite for designing and selection of an effective and safe permeation enhancer. The drug primarily uses one route and may switch to the other route if properties of that route are changed considerably by a penetration enhancer.

Among the various classes of permeation enhancers, chitosan and its derivatives are having favorable biological properties in terms of biocompatibility, nontoxicity, and biodegradability apart from good permeation enhancement effects. Because of their relatively less and reversible effects on the epithelial morphology, chitosan and its derivatives are promising and unique bioenhancers for mucosal delivery of various therapeutic agents. It is generally considered that oral mucosa is more resistant to damage than other mucosal membranes. The oral mucosal damage caused by bile salts would be considered as temporary and reversible and less serious than the nasal mucosal damage.

In case of in vitro permeation studies, maintaining and assessing the integrity and viability of the excised tissue is of paramount importance before and during the experiment. CLSM and MTT assay can be successfully employed to accurately and reliably determine the viability of buccal mucosa. The viability of the tissue can be better maintained in KBR than in PBS at 34°C[192]. It has also been reported that MTT assay can be a reliable method for toxicity screening purposes[193].

Mechanical methods of mucosal permeation enhancement, such as iontophoresis, electroporation, sonophoresis, and electro-osmosis, are also investigated and are under extensive research, but their consistent and optimum use has not been established.

To optimize the concentration of enhancer to limit its toxicity while facilitating an enhancing effect reproducibly will be a big challenge for future developments. Current research should be focused on developing a penetration enhancer, especially for buccal drug delivery, that is rapidly reversible in action and if possible, without membrane toxicity (i.e., selective only against the target cells and inert with respect to cells participating in irritation). Developments in the polymer science might provide an alternative to design a novel, nontraditional permeation enhancer having excellent permeation enhancement properties without causing irreversible toxic and damaging effects to the membrane.

Advances in permeability modulation and formulation with appropriate enhancers can provide for effective and feasible buccal drug delivery for many drugs, which otherwise have to be injected or ingested with water.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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