



Review Article

**IN-VITRO AND IN-VIVO EVALUATION OF NOVEL MUCOADHESIVE BUCCAL DRUG
DELIVERY SYSTEMS-A REVIEW**

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Abstract: The buccal mucosa is one of the administration sites that might provide an alternative for peroral administration. Mucoadhesive buccal drug delivery systems is one of the most important novel drug delivery systems with its various advantages and it has a lot of potential in formulating dosage forms for various chronic diseases. The delivery of drugs through the buccal mucosa has attracted much research interest over the past two decades and numerous approaches, both conventional and complex, have been developed in an attempt to deliver a variety of pharmaceutical compounds via the buccal route. Buccal drug delivery helps to bypass first- pass metabolism by allowing direct access to the systemic circulation through the internal jugular vein. The current review provides a good insight idea anatomy and nature of oral cavity, the design of appropriate in vitro and in vivo methods to evaluate the behaviour of these bioadhesive delivery systems. The development of a range of in vitro methods is outlined including disintegration/dissolution testing, in vitro mucoadhesion tests, in vitro residence time evaluation and permeability studies.

Keywords: In vitro and in vivo modelling, buccal mucosa, evaluation techniques

Introduction

Adhesion as a process is simply defined as the “fixing” of two surfaces to one another¹. There are many different terminological subsets of adhesion depending upon the environment in which the process occurs. When adhesion occurs in a biological setting it is often termed “bioadhesion”, further more if this adhesion occurs on mucosal membranes it is termed “mucoadhesion”. Bioadhesion can be defined as the binding of a natural or synthetic polymer to a biological substrate. When this substrate is a mucous layer, the term mucoadhesion is often used². Mucoadhesion has been widely promoted as a way of achieving site-specific drug delivery through the incorporation of mucoadhesive hydrophilic polymers within pharmaceutical formulations along with the active pharmaceutical ingredient (API). The rationale being that the formulation will be ‘held’ on a biological surface for localised drug delivery. The API will be released close to the site of action with a consequent enhancement of bioavailability³.

Drug delivery via the buccal route, using bioadhesive dosage forms offers such a novel route of drug administration. Buccal mucosa has excellent accessibility, an expanse of smooth muscle and relatively immobile mucosa, hence suitable for administration of retentive dosage forms. Direct access to the systemic circulation through the internal jugular vein bypasses drugs from the hepatic first pass metabolism leading to high bioavailability.

Mucoadhesive drug delivery systems includes the following⁴

- Buccal delivery system
- Oral delivery system
- Vaginal delivery system
- Rectal delivery system
- Nasal delivery system
- Ocular delivery system

There are several advantages in using bio/mucoadhesive drug delivery systems:

- (1) As a result of adhesion and intimate contact, the formulation stays longer at the delivery site improving API bioavailability using lower API concentrations for disease treatment.
- (2) The use of specific bioadhesive molecules allows for possible targeting of particular sites or tissues, for example the gastrointestinal (GI) tract.
- (3) Increased residence time combined with controlled API release may lead to lower administration frequency.
- (4) The avoidance of first-pass metabolism.
- (5) Additionally significant cost reductions may be achieved and dose-related side effects may be reduced due to API localisation at the disease site³.

Buccal mucosal structure, function and composition and its suitability

The oral cavity is lined with mucous membranes with a total surface area of 100 cm². It is possible to observe several distinct areas: the floor of mouth (sublingual), the

buccal mucosa (cheeks), the gums (gingiva), the palatal mucosa and the lining of the lips.

Mucus is a complex viscous adherent secretion which is synthesized by specialized goblet cells. These goblet cells are glandular columnar epithelium cells and line all organs that are exposed to the external environment. The thickness of the buccal mucosa is measured to be 500–800 μm and is rough textured, hence suitable for retentive delivery systems⁵. The turnover time for the buccal epithelium has been estimated at 5–6 days⁶. Buccal mucosa composed of several layers of different cells as shown in (Fig 1) The epithelium is similar to stratified squamous epithelia found in rest of the body and is about 40–50 cell layers thick. Lining epithelium of buccal mucosa is the nonkeratinized stratified squamous epithelium that has thickness of approximately 500–600 μ and surface area of 50.2 cm^2 . Mucus is composed mainly of water (>95%) and mucins, which are glycoprotein's of exceptionally high molecular weight (2–14 _ 106 g/mol). Also found within

this “viscoelastic soup” are proteins, lipids and muco polysaccharides, which are found in smaller proportions (<1%). The mucin glycoproteins form a highly entangled network of macromolecules that associate with one another through non-covalent bonds. Such molecular association is central to the structure of mucus and is responsible for its rheological properties. Furthermore, pendant sialic acid ($\text{pK}_a = 2.6$) and sulphate groups located on the glycoprotein molecules result in mucin behaving as an anionic polyelectrolyte at neutral pH⁷.

The exact composition of mucus may vary with the site of secretion, its physiological or mechanical role, and the presence of any underlying disease state⁸. One particular point of interest is the strategic position of mucus in many disease processes in which the interactions of epithelial cells and their surroundings have gone astray such as is seen in inflammatory and infectious diseases, cancer and metastasis⁹. Such scenarios may allow a means of targeting therapeutics to such conditions more effectively.

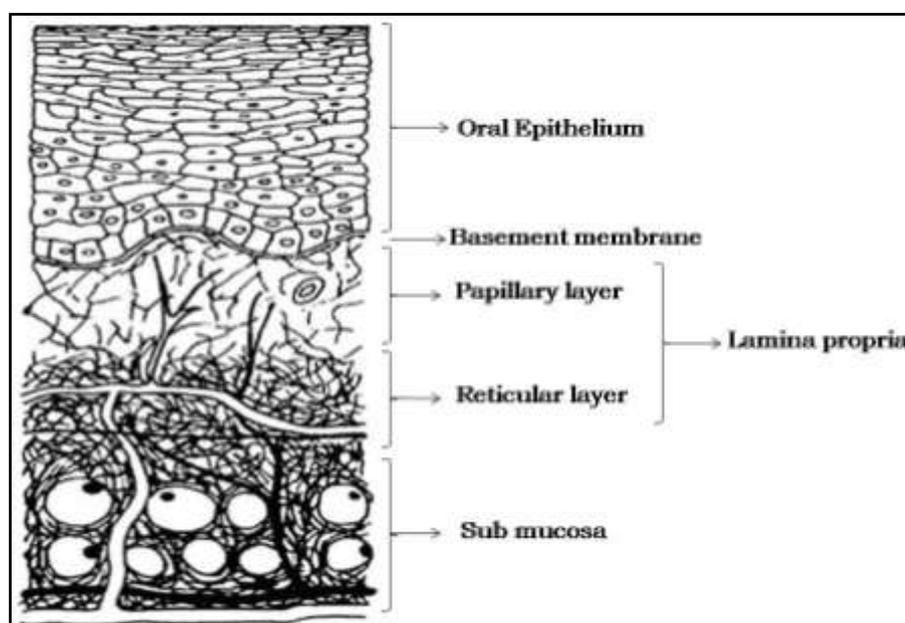


Figure 1: Cross-section of buccal mucosa

Mucoadhesion mechanism and theories of polymer attachment

As stated, mucoadhesion is the attachment of the drug along with a suitable carrier to the mucous membrane. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains. Mechanism of polymer attachment to mucosal surface in the buccal cavity are not yet fully understood, but certain theories of Bioadhesion suggested that it may occur via physical entanglement (diffusion theory), chemical interaction, such as electrostatic, hydrophobic, hydrogen bonding and vanderwaal's interactions (absorption and electronic theories). However, most research has described bio-adhesive bond formation as a three step process.

Step 1: Intimate contact between a bioadhesive and a membrane (wetting or swelling phenomenon)¹⁰

Step 2: penetration of the bioadhesive into the tissue or into the surface of the mucous membrane (interpenetration)¹¹

Step 3: Formation of chemical bonds between the entangled chains¹².

Mucoadhesive polymer drug delivery platforms

Polymer is a generic term used to describe a very long molecule consisting of structural units and repeating units of monomers connected by covalent chemical bonds. The polymeric attributes that are pertinent to high levels of retention at applied and targeted sites via mucoadhesive bonds include hydrophilicity, negative charge potential and the presence of hydrogen bond forming groups. Additionally, the surface free energy of the polymer should be adequate so that ‘wetting’ with the mucosal surface can be achieved. The polymer should also possess sufficient flexibility to penetrate the mucus network, be biocompatible, non-toxic and economically favourable¹³. The polymers that are commonly employed in the manufacture of mucoadhesive drug delivery platforms that

adhere to mucin–epithelial surfaces may be conveniently divided into three broad categories ¹⁴

- (1) Polymers that become sticky when placed in aqueous media and owe their bioadhesion to stickiness.
- (2) Polymers that adhere through non-specific, non-covalent interactions those are primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant).
- (3) Polymers that bind to specific receptor sites on the cell surface

Hydrophilic polymers: It contains carboxylic group and possess excellent mucoadhesive properties. These are PVP (poly vinyl pyrrolidone), Mc (methyl cellulose), Scmc (sodium carboxy methyl cellulose), HPC (hydroxyl propyl cellulose) ¹⁵

Hydrogels: These swell when in contact with water and adhere to the mucus membrane. These are further classified according to their charge

Anionic polymers - carbopol, polyacrylates

Cationic polymers - Chitosan

Neutral/non ionic polymers- eudragit analogues

They can also be classified as

Synthetic polymers

Natural polymers

Synthetic polymers - Cellulose derivatives, Carbopol, etc.

Natural polymers - Tragacanth, Pectin, gelatin, Sodium alginate, acacia.

Methods for measuring mucoadhesive potential

These tests are important during the design and development of a mucoadhesive release system to study compatibility, stability, surface analysis and bioadhesive bond strength. Several dosage forms have been developed and explored to enable drug delivery through the oral mucosa and include liquids (solutions, suspensions), semi-solids (gels, creams, ointments, and emulgels), solids (tablets, lozenges, films, wafers, patches, microparticles) and sprays ¹⁶. Regardless of the type of the dosage form, in vitro and in vivo methods are required for routine quality control or formulation development and include evaluation of drug release, permeation and often mucoadhesion. (Table 1) list the important in vitro and in vivo methods available for the assessment of buccal dosage forms.

Disintegration test

In vitro disintegration tests are usually performed for rapid dissolving films and tablets to determine the disintegration rate ^{17,18} when they come in contact with the mucus and saliva. The changes in integrity of such dosage forms can also be assessed visually when performing dissolution studies. The USP disintegration test employs purified water as a disintegrating medium which is far from the physiological conditions that prevail in the oral cavity (Table 2) ^{19,20}. Attempts have been made to develop disintegration tests that better mimic the in vivo conditions of the oral cavity.

Table 1Types of buccal dosage forms and their testing requirements

Test methods	Tablets/ lozenges	Films/wafers/ Patches	Liquids/gel/cream/ ointments	Spray
Weight variation	✓	✓		
Uniformity of content	✓	✓	✓	✓
Friability	✓			
Resistance to crushing	✓			
Tensile strength	✓	✓		
Thickness	✓	✓		
Film endurance		✓		
Viscosity			✓	
Droplet size				✓
Disintegration Test	✓	✓		
Dissolution test	✓	✓	✓	
Residence time	✓	✓	✓	
Mucoadhesion Strength	✓	✓	✓	
Permeability study	✓	✓	✓	✓
Buccal absorption test	✓	✓	✓	✓
Perfusion study	✓	✓	✓	✓
Pharmacokinetic study	✓	✓	✓	✓

Table 2 Physiological conditions of human oral cavity

Parameters	Value
pH of saliva	5.5 to 7.0
Volume of saliva (continuous available volume)	696±312 µl
Viscosity of saliva (mPa.s) (n=40)	1.09±0.11
Protein in saliva (n=40) (mg/ml)	0.70±0.30
Amylase in saliva (n=40) (IU/ml)	325±199

Determination of peel strength

The peel adhesion tests are mainly used for buccal and transdermal patches²¹. The test is based on the calculation of energy required to detach the dosage form. From the substrate material (usually excised buccal mucosa) attached through the bioadhesive material in the direction as shown in (Fig 2).

Peel work is the sum of the following components

- Surface energy that results from the creation of two free surfaces (energy of de wetting) also referred to as the intrinsic work of adhesion Bulk energy that dissipates into the stripping member
- Strain energy in the newly detached strip

Intrinsic work of adhesion is independent of the following:

- Peel rate
- Peel angle
- Thickness of the stripping member

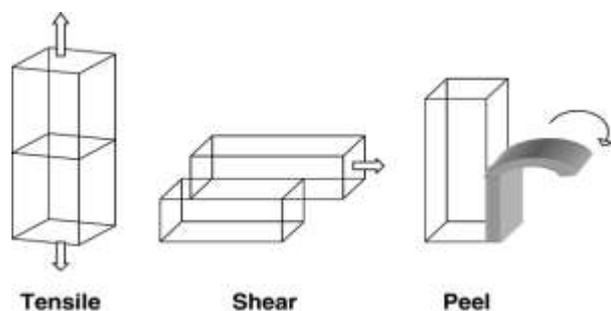


Figure 2: Representation of peel, shear and tensile forces

Values of intrinsic work of adhesion vary from 0.07 J/m squared for hydrocarbon vander Waal's interactions, 2 J/m squared for a system with covalent bonding as part of the adhesion. The work of fracture can be several orders of magnitude greater than the intrinsic work of adhesion²².

Determination of shear strength

Shear stress, τ is the force acting tangentially to a surface divided by the area of the surface. It is the force per unit area required to sustain a constant rate of fluid movement. Mathematically, shear stress can be defined as:

$$\tau = F/A$$

where, τ shear stress, F force, A area of the surface subjected to the force.

If a fluid is placed between two parallel plates spaced 1.0 cm apart, and a force of 1.0 dyn is applied to each square centimetre of the surface of the upper plate to keep it in motion, the shear stress in the fluid is 1 dyn/cm² at any point between the two plates²³⁻²⁵.

Determination of tensile strength

Tensile stress is also termed Maximum Stress or Ultimate Tensile Stress. The resistance of a material to a force tending to tear it apart, measured as the maximum tension the material can withstand without tearing. Tensile strength can be defined as the strength of material expressed as the greatest longitudinal stress it can bear without tearing apart. As it is the maximum load applied in breaking a tensile test piece divided by the original cross-sectional area

of the test piece, it is measured as Newtons /sq.m. Specifically, the tensile strength of a material is the maximum amount of tensile stress that it can be subjected to before failure. The definition of failure can vary according to material type and design methodology.

There are three typical definitions of tensile strength:

- Yield Strength — The stress a material can withstand without permanent deformation.
- Ultimate Strength — The maximum stress a material can with stand.
- Breaking Strength — The stress coordinate on the stress – strain curve at the point of rupture.

Methods using the tensile strength usually measure the force required to break the adhesive bond between a model membrane and the test polymers²⁴. determined tensile strength of flat-faced buccal adhesive tablets, with a diameter of 5.5 mm containing 50 mg of the mucoadhesive material is to be tested for its shear stresses by clamping the model mucosal surface between two plates, one having a U-shaped section cut away to expose the test surface. The tablet was attached to a Perspex disc, and then placed into contact with the exposed mucosa at the base of the U shaped cut. 1.5 g weight was used to consolidate the adhesive joint for 2min, and the plates were oriented from horizontal to vertical and Perspex disc attached to the underside of the balance, which was linked to a microcomputer for data collection.

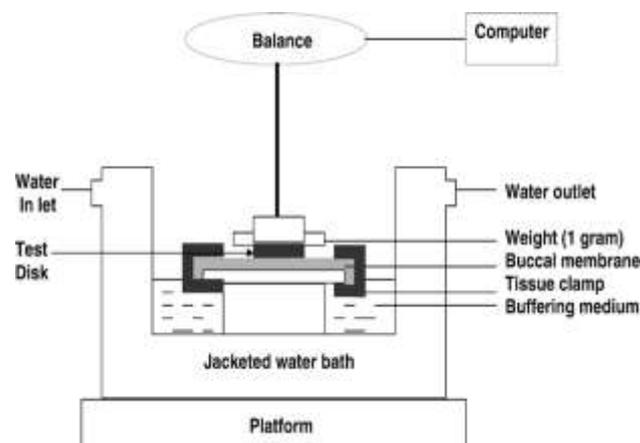


Figure 3: Schematic representation of apparatus for measuring tensile strength

Colloidal gold staining method

The technique²⁶ employs red colloidal gold particles, which were adsorbed on mucin molecules to form mucin-gold conjugates, which upon interaction with bioadhesive hydrogels develops a red color on the surface. This can be quantified by measuring at 525 nm either the intensity on the hydrogel surface or the conjugates.

Direct staining method

It is a novel technique to evaluate polymer adhesion to human buccal cells following exposure to aqueous polymer dispersion²⁷, both in vitro and in vivo. Adhering polymer was visualized by staining with 0.1% w/v of either Alcian blue or Eosin solution; and the

uncomplexed dye was removed by washing with 0.25M sucrose. The extent of polymer adhesion was quantified by measuring the relative staining intensity of control and polymer treated cells by image analysis. Carbopol 974 P, polycarbophil and chitosan were found to adhere to human buccal cells from 0.10% w/w aqueous dispersions of these polymers.

Analytical ultracentrifuge criteria for mucoadhesion

These methods are useful in identifying the material that is able to form complexes with the mucin. The assay can be done for change in molecular mass using sedimentation equilibrium, but this has an upper limit of less than 50MDa. Since complexes can be very large, a more sensible assay procedure is to use sedimentation velocity with change in sedimentation coefficients, as their marker for mucoadhesion. Where mucin is available in only miniscule amounts, a special procedure known as sedimentation fingerprinting can be used for assay of the effect on the mucoadhesive. UV absorption optics is used as the optical detection system. However, in this case the mucoadhesive is invisible, but the pig gastric mucin at the concentrations normally employed is visible. The sedimentation ratios (complex/mucin), the ratio of the sedimentation coefficient of any complex involving the mucin to that of pure mucin itself, is used as the measure for mucoadhesion²⁸.

Atomic force microscopy

This method is based on the changes in surface topography when the polymer bound on to buccal cell surfaces. Unbound cells shows relatively smooth surface characteristics with many small craters like pits and indentations spread over cell surfaces, while polymer bound cells will lose crater and indentation characteristics and gained.

Electrical conductance

The method is used modified rotational viscometer to determine electrical conductance of various Semi-solid mucoadhesive ointments and found that the electrical conductance was low in the presence of adhesive material.

Fluorescent probe method

In this method the membrane lipid bilayered and membrane proteins were labelled with pyrene and fluoresce in isothiocyanate, respectively. The cells were mixed with the mucoadhesive agents and changes in fluorescence spectra were monitored. This gave a direct indication of polymer binding and its influence on polymer adhesion²⁹.

Lectin binding inhibition technique

The method involves an avidin–biotin complex and a colorimetric detection system to investigate the binding of bioadhesive polymers to buccal epithelial cells without having to alter their physicochemical properties by the addition of marker entities. The lectin can canavalian A has been shown to specifically bind to sugar groups present on the surface of buccal cells. If polymers bind to buccal cells, they will mask the surface glycoconjugate, thus reducing or inhibiting cancanavalian a binding³⁰.

Thumb test

This is a very simple test used for the qualitative determination of peel adhesive strength of the polymer and is useful tool in the development of buccal adhesive delivery systems. The adhesiveness is measured by the difficulty of pulling the thumb from the adhesive as a function of the pressure and the contact time. Although the thumb test may not be conclusive, it provides useful information on peel strength of the polymer.

Rheological measurement of mucoadhesion

The rheological profiling of polymer–mucus mixtures can provide an acceptable in vitro model representative of the true in vivo behaviour of a mucoadhesive polymer. The mucoadhesive potential of polymer can be determined rheologically comparing binary polymer/mucus blends to the rheological sum of similarly concentrated mono component mucus and polymer systems.^{31,32}

Viscometric method

These methods are useful to quantify mucin–polymer bioadhesive bond strength. Viscosities of 15% w/w porcine gastric mucin dispersion were measured with Brookfield's viscometer. In absence or presence of selected neutral, anionic and cationic polymer, viscosity components and the forces of bioadhesion were calculated. The observed a positive rheological synergism when chitosan solutions prepared in pH 5.5 acetate buffers and in 0.1 M HCl, were mixed with a fixed amount of porcine gastric mucin. The mixtures with mucin showed a viscosity greater than the sum of polymer and mucin viscosities.

Swelling studies

Buccal adhesive dosage forms were weighed individually (w1) and placed separately in Petri Dishes containing 4ml of phosphate buffer ph 6.6. at regular intervals (.5, 1,2,3,4,5,6 hours) the dosage forms were removed from the Petri dishes and excess surface water was removed using filter paper. The dosage form were reweighed and swelling index (SI) was calculated as follows,³³

$$SI = (W2 - W1) / W1 \times 100$$

Surface pH

As an acidic or alkaline pH may cause irritation to the buccal mucosa, were necessary to keep the surface pH as close to neural as possible⁶. The tablet was allowed to swell by keeping it in contact with 1 ml of distilled water and allowed to swell for 1-2 h at room temperature. The pH was measured by bringing the electrode in contact with the surface of tablets and allowing equilibrating for 1 min³⁴. The mean of three reading was recorded.

Measurement of force of attachment

The adhesive strength at a bonding interface can be measured by measuring the force required to detach one entity from the other through the application of an external force. As such the destruction of the adhesive bond is usually under the application of either a shearing, tensile or peeling force. The determination of such a force of attachment was investigated by Smart et al. Here the authors

used a modified version of the Wilhelmy plate surface technique in order to determine mucoadhesion of a range of polymers (Fig.8). The device basically consists of a glass plate (which is laden with the polymer to be studied) suspended from a microbalance. The polymer-coated plate was then slowly dipped into a beaker of mucus. The work required to remove the various polymer coated glass slides could then be related to one another and their adhesiveness could be ranked.^{35,36} The lack of biological tissue in such a setup may not represent true mucoadhesion. Most of the mucoadhesive delivery systems will tend to exhibit other mechanical forces, such as the shear stresses exhibited within the buccal cavity. The effect of both these important forces on measuring the adhesive bond was made possible via the use of a dual tensiometer.

Permeability studies

Drug permeability models are usually employed to determine the barrier nature of different biological tissues where by the diffusion of drugs can be studied in a controlled environment in which variables such as temperature, pH and osmolarity can be easily controlled³⁷. Human buccal tissues may be the most useful to mimic the real in vivo situation but because of their limited availability researchers generally use animal tissue. However, such data should be treated with caution because of the differences observed between human and animal buccal mucosa as detailed below.

Animal buccal tissue

The use of animal buccal epithelium as a model for its human counterpart for the purpose of predicting trans buccal drug absorption kinetics is reported to be acceptable because of their similarity in the nature of keratinization, thickness and lipid composition. Rats, hamster, rabbit, dog and primate tissue are those most frequently used in such permeation studies and a comparison with human buccal mucosa is provided in Table 4. The oral mucosa of rats^{38,39} and hamsters^{40,41} has been used in several studies though the mucosa derived from these animals is keratinized as opposed to the non-keratinized nature of human buccal mucosa. Rabbit buccal mucosa is non-keratinized in parts and as such has also been used in some in vitro studies, however, identification and isolation of this area of tissue as opposed to the keratinized regions, limit the amount of suitable tissue available and can be unreliable. Therefore, buccal tissue from other animal species in particular that of monkeys dogs, and pigs has been extensively used for in vitro drug permeability studies. Although the buccal mucosa of the dog and monkey is non keratinized, the epithelium of the mucosa in these animals is much thinner, and more permeable than that of humans. As such the buccal tissue of pigs is the most frequently utilized model due to its relative similarity to human tissue⁴², ethical considerations and the low cost associated with tissue acquisition. In most permeation studies, animals are sacrificed immediately before the start of an experiment, the buccal mucosa with underlying connective tissue is excised from the oral cavity, the connective tissue is surgically removed, and the buccal mucosal membrane is isolated.

Table 3: Comparison of buccal mucosa of different mammals⁴³⁻⁴⁵.

Models	Tissue structure	Buccal membrane thickness (μm) (mean \pm SD)	Permeability constant for tritiated water ($\times 10^7$ cm/min) (mean \pm SD)
Human	Non-keratinized	580 \pm 90	579 \pm 122
Dog	Non-keratinized	126 \pm 20	1045 \pm 37
Hamster	Keratinized	115.3 \pm 11.5	Not available
Pig	Non-keratinized	772 \pm 150	634 \pm 60
Rabbit	Partially keratinized	600	Not available
Rat	Keratinized	Not available	Not available
Monkey	Non-keratinized	271 \pm 50	1025 \pm 154

Diffusion cells

The most widely used in vitro methodology to study oral mucosal permeability is by testing drug permeation across isolated mucosal tissue mounted in permeability chambers. The use of diffusion cells makes it possible to determine the actual amount of drug that diffuses across the mucosal barrier as well as the rate of drug diffusion. Most commonly two types of permeability cells have been used: vertical (Franz diffusion cell) and side-by-side horizontal (Using chambers)⁴⁶.

The Franz diffusion chamber is a static, one-chamber diffusion cell where compound in neat solution or in formulated form is applied to the mucosal apical surface, which faces the air through the open cell cap that constitutes the donor compartment. The receiver chamber volume is

typically 6–8 ml and a stirring bar maintains homogeneous temperature and mixes the solution in the receiver compartment. The temperature of the apparatus is maintained at 37°C by placing the diffusion cell into a heated water bath. Samples from the receiver solution are taken via the side port on the diffusion cell typically for up to 180 min, a time period much shorter (few hours) than that at which isolated buccal mucosa is reportedly viable in this system. A major hurdle for using static diffusion cells for a permeation study is the limited volume of receiver fluid which may influence the maintenance of sink conditions especially with poorly soluble drugs. The addition of a co-solvent such as polyethylene glycol, methanol or ethanol in receiver fluid, or the use of surfactant are the most common approaches adopted in the literature to overcome this⁴⁷.

However, the implication of using co-solvents on the permeability of the mucosa needs to be noted. Modification to the existing static diffusion method in order to mimic the salivary flow in the oral cavity and also to overcome the difficulty associated with maintaining sink conditions has resulted in the use of flow through cells for buccal permeability studies⁴⁸. Although similarities exist between the two methods in Franz diffusion cells, the buccal mucosal surface is exposed to air which can result in drying of tissue and potential tissue death. While in flow through cells the receiver solution flows underneath the buccal mucosa, which ensures that there is no accumulation of compound in the receptor compartment. Modified Using chambers have also been commonly used to assess the transport of many compounds across buccal mucosa^{49, 50}. Using chambers consist of two half chambers which when clamped together are separated by a piece of biological tissue. The advantages associated with this type of diffusion cell is that the donor and receptor chambers are provided with carbogen gas (95% of O₂ and 5% of CO₂), which not only provides stirring but also helps to maintain the viability of the buccal tissue⁵¹.

Dissolution studies

The comparison of different oral trans mucosal products or evaluation of newly developed trans mucosal formulations is problematic due to the lack of standardized guidance available in the pharmacopeia. In the specification, the USP dissolution apparatus II is used at a paddle speed of 50 rpm with 900 ml water as dissolution medium which does not correlate with the amount of saliva available for in vivo dissolution and thus may not produce results that reflect in vivo dissolution^{52, 53}. Several studies have been performed to investigate drug dissolution in smaller volumes or using different apparatus.

Perfusion studies

Perfusion cells have been proposed and used to study the regional absorption of drugs in the oral cavity and to overcome the limitation of nonspecific absorption across all surfaces of the oral cavity encountered by the buccal absorption test. Perfusion cells have been designed in such a way that it can be clamped or attached to particular mucosa within the oral cavity of both animals and humans^{54, 55}. In

general, perfusion experiments are performed by attaching the perfusion chambers to the oral mucosa of an anesthetized animal model. Drug solution is circulated through the device and collected at different time intervals. Blood samples are withdrawn periodically during the experiments to generate the pharmacokinetic data. When plasma cannot be assayed, the drug concentration in saliva can be measured and this information is used to extrapolate the plasma concentration. Such a method was recently used to assess the buccal absorption of nicotine in humans where the disappearance of nicotine from the perfusion solution was used to determine the rate of nicotine absorption. Saliva was simultaneously collected directly from the parotid gland using a modified Carlson–Crittenden cup as a surrogate for plasma. Several limitations⁵⁶ are associated with this method such as the possibility of leakage and large inter-subject variation. This approach works on the principle of pharmacodynamic activity of the drug and hence it would be useful for those drugs whose bioavailability is measured via its pharmacodynamic properties where their biological response is proportional to drug concentration. Also local drug metabolism in the tissue is another factor that needs to be considered.

Pharmacokinetic studies

Buccal absorption and perfusion studies usually provide an insight into the ability of a formulation to deliver a drug but these lack real in vivo systemic evaluation and other physiological factors which may contribute to overall performance of the dosage forms. Hence it is often essential to perform an in vivo pharmacokinetic study. Rabbit⁵⁷, dog and pig are the animal models that have been used so far to perform such initial in vivo studies prior to human assessment. There are several other studies reported in the literature using animals which are detailed in Table 6 including their clinical outcomes. However the ideal animal model for performance evaluation of buccal drug delivery systems remains in doubt and further research is required before any further conclusions can be drawn. Human volunteers are also frequently used to test the pharmacokinetic profiles of buccal delivery systems and provide the most realistic evaluation of their in vivo performance.

Table 4 Examples of in vivo evaluation of buccal drug delivery system

Drug	Primary aim	Dosage forms	In vivo model	Clinical outcomes	References
Fentanyl citrate	Bioavailability studies of different Formulation	Tablet and lozenge	Human	The rate and extent of fentanyl absorption were greater following administration of a tablet compared to lozenge formulation. An approximately 30% smaller dose of tablet achieved systemic exposure comparable to lozenge	58
Prochlorperazine	Comparison of buccal and oral administration	Tablet	Human	Buccal prochlorperazine achieved a significantly faster onset of effect compared with oral prochlorperazine (p=0.04), and was significantly better in reducing the frequency of nausea (p=0.02) and severity of vomiting	59

				(p=0.07) at 24–36 h. The frequency of vomiting was also reduced by buccal prochlorperazine compared with oral prochlorperazine, but this difference was only of borderline significance (p=0.07).	
Chlorpheniramine maleate	Delivery through mucoadhesive patch	Patch	Human	T _{max} values were higher for buccal administration than the oral administration and the difference was statistically significant (p<0.05). The overall mean AUC by buccal route was 1.46 times higher than that of oral route and the difference was found statistically significant (p<0.05) demonstrating improved bioavailability from buccal patch.	60
Lidocaine hydrochloride	Novel mucoadhesive drug Delivery	Patch	Rabbits	Lidocaine hydrochloride was maintained in the plasma for at least 6 h and had a plasma concentration above the therapeutic level (2 µg/ml) therefore it was believed to be effective for overnight therapy. Patches were well adhered to buccal mucosa of the rabbits over the 8 h study period.	61
Etomidate	Feasibility study of buccal Administration	Lozenges	Dog	Approximately 90% or more of the absorption via buccal mucosa took place during the period in which the drug was in contact with the mucosa (15 min). Absolute bioavailability of 13.6±10.7% was achieved through buccal administration for etomidate.	62

In vitro–in vivo correlation for buccal drug delivery systems

Correlations between in vitro and in vivo data (IVIVC) are often used during pharmaceutical development in order to optimize the formulation while reducing product development time and costs. A good correlation is a tool for predicting in vivo results based on in vitro data and it allows dosage form optimization with the fewest possible trials in man, fixes drug release acceptance criteria, and can be used as a surrogate for further bioequivalence studies⁶³.

Conclusion

Buccal drug delivery has attracted significant attention due to its distinct features such as avoidance of first pass metabolism, improving medication compliance and rapid drug response. Buccal drug delivery validated in vitro and in vivo methods are essential tools to assess the performance of buccal drug delivery systems and to predict there in vivo behavior. However, the lack of standardized methodologies for the systematic evaluation of buccal drug delivery systems is apparent. Mucoadhesive dosage forms extend from the simple oral mucosal delivery to the nasal, vaginal, ocular and rectal drug delivery systems. Significant work is still needed to establish an appropriate in vitro model or models which can closely resemble the in vivo condition and can better predict the performance of a drug delivery system in the clinical setting.

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