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Review on Buccal Adhesive Drug Delivery System: A Promising Strategy for Poorly Soluble Drugs

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ABSTRACT

Rapid developments in the field of molecular biology and gene technology resulted in generation of many macromolecular drugs including peptides, proteins, polysaccharides and nucleic acids in great number possessing superior pharmacological efficacy with site specificity and devoid of untoward and toxic effects. However, the main impediment for the oral delivery of these drugs as potential therapeutic agents is their extensive presystemic metabolism, instability in acidic environment resulting into inadequate and erratic oral absorption. Parentral route of administration is the only established route that overcomes all these drawbacks associated with these orally less/inefficient drugs. But, these formulations are costly, have least patient compliance, require repeated administration, in addition to the other hazardous effects associated with this route. Over the last few decades' pharmaceutical scientists throughout the world are trying to explore transdermal and transmucosal routes as an alternative to injections. Among the various transmucosal sites available, mucosa of the buccal cavity was found to be the most convenient and easily accessible site for the delivery of therapeutic agents for both local and systemic delivery as retentive dosage forms, because it has expanse of smooth muscle which is relatively immobile, abundant vascularization, rapid recovery time after exposure to stress and the near absence of langerhans cells. Direct access to the systemic circulation through the internal jugular vein bypasses drugs from the hepatic first pass metabolism leading to high bioavailability. Further, these dosage forms are self-administrable, cheap and have superior patient compliance. Developing a dosage form with the optimum pharmacokinetics is a promising area for continued research as it is enormously important and intellectually challenging. With the right dosage form design, local environment of the mucosa can be controlled and manipulated in order to optimize the rate of drug dissolution and permeation. A rational approach to dosage form design requires a complete understanding of the physicochemical and biopharmaceutical properties of the drug and excipients. Advances in experimental and computational methodologies will be helpful in shortening the processing time from formulation design to clinical use. This paper aims to review the developments in the buccal adhesive drug delivery systems to provide basic principles to the young scientists, which will be useful to circumvent the difficulties associated with the formulation design.

Keywords: Buccal Drug Delivery, Bioadhesive, Natural Polymers, Formulation, Permeation enhancers and Evaluation Studies.

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I. INTRODUCTION

The main impediment to the use of many hydrophilic macro-molecular drugs as potential therapeutic agents is their inadequate and erratic oral absorption. The relatively recent evolution of recombinant DNA research and modern synthetic and biotech- nological methodologies allow the biochemist and chemist to produce vast quantities of variety of peptides and proteins possessing better pharmacological efficacy. However, therapeutic potential of these compounds lies in our ability to design and achieve effective and stable delivery systems. The future challenge of pharmaceutical scientists will not only be polypeptide cloning and synthesis, but also to develop effective non parenteral delivery of intact proteins and peptides to the systemic circulation. Based on our current understanding of biochemical and physiological aspects of absorption and metabolism of many bio-technologically-produced drugs, they cannot be delivered effectively through the conventional oral route. Because after oral administration many drugs are subjected to presystemic clearance extensive in liver, which often leads to a lack of significant correlation between membrane permeability, absorption, and bioavailability [1]. Difficulties associated with parenteral delivery and poor oral availability provided the impetus for exploring alternative routes for the delivery of such drugs. These include routes such as

pulmonary, ocular, nasal, rectal, buccal, sublingual, vaginal, and transdermal. In absence of external stimuli to facilitate absorption, use of these alternative routes has had limited success. Various strategies have been implemented to promote the bioavailability of these drugs, including supplemental administration of enzyme inhibitors, use of absorption enhancers, novel formulation strategies, and reversible chemical modifications [2].

Among the various transmucosal routes, 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. Other advantages such as low enzymatic activity, suitability for drugs or excipients that mildly and reversibly damages or irritates the mucosa, painless administration, easy drug withdrawal, facility to include permeation enhancer/enzyme inhibitor or pH modifier in the formulation and versatility in designing as multidirectional or unidirectional release systems for local or systemic actions etc, opts buccal adhesive drug delivery systems as promising option for continued research [3] .

Buccal mucosal structure and its suitability

Buccal region is that part of the mouth bounded anteriorly and laterally by the lips and the cheeks, posteriorly and medially by the teeth and/or gums, and above and below by the reflections of the mucosa from the lips and cheeks to the gums. Numerous racemose, mucous, or serous glands are present in the sub mucous tissue of the cheeks [4]. The buccal glands are placed between the mucous membrane and buccinator muscle: they are similar in structure to the labial glands, but smaller. About five, of a larger size than the rest, are placed between the masseter and buccinator muscles around the distal extremity of the parotid duct; their ducts open in the mouth opposite the last molar tooth. They are called molar glands [5] . Maxillary artery supplies blood to buccal mucosa and blood flow is faster and richer (2.4 ml/min/cm^2) than that in the sublingual, gingival and palatal regions, thus facilitates passive diffusion of drug molecules across the mucosa. The thickness of the buccal mucosa is measured to be 500–800 μm and is rough textured, hence suitable for retentive delivery sys- terms [6] . The turnover time for the buccal epithelium has been estimated at 5–6 days [7]. 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 [5]. 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. Basement membrane, lamina propria followed by the submucosa is present below the epithelial layer [8]. Lamina propria is rich with blood vessels and capillaries that open to the internal jugular vein. Lipid analysis of buccal tissues shows the presence of phospholipid 76.3%, glu- cosphingolipid 23.0% and ceramide NS at 0.72%. Other lipids such as acyl glucosylated ceramide, and ceramides like Cer AH, Cer AP, Cer NH, Cer AS, and EOHP/NP are completely absent [9]. The primary function of buccal epithelium is the protection of the underlying tissue. In nonkeratinized regions, lipid-based permeability barriers in the outer epithelial layers protect the underlying tissues against fluid loss and entry of potentially harmful environmental agents such as antigens, carcinogens, microbial toxins and enzymes from foods and beverages [10].

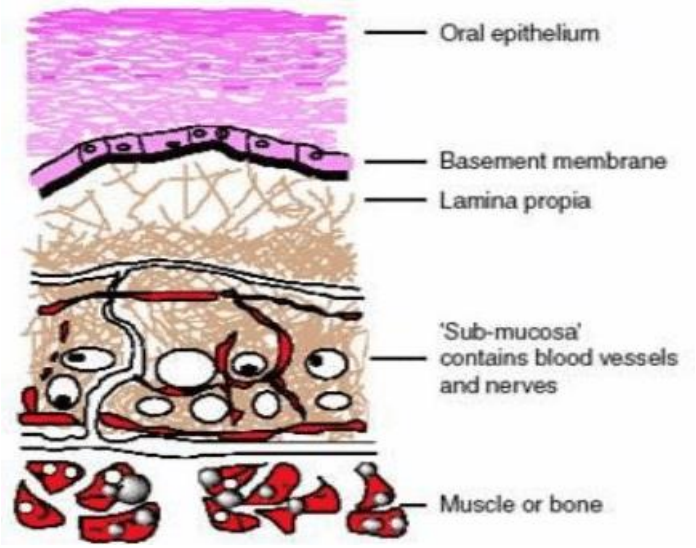

Figure 1 Cross-section of buccal mucosa

Absorption pathways

Studies with microscopically visible tracers such as small proteins [11] and dextrans [12] suggest that the major pathway across stratified epithelium of large molecules is via intercellular spaces and that there is a barrier to penetration as a result of modifications to the intercellular substance in the superficial layers. However, rate of penetration varies depending on the physicochemical properties of the molecule and the type of tissue being traversed. This has led to the suggestion that materials uses one or more of the following routes simultaneously to cross the barrier region in the process of absorption, but one route is pre- dominant over the other depending on the physicochemical pro- perties of the diffusant [13].

Passive diffusion

Transcellular or intracellular route (crossing the cell membrane and entering the cell)

Paracellular or intercellular route (passing between the cells)

Carrier mediated transport

Endocytosis

The flux of drug through the membrane under sink condition for paracellular route can be written as Eq. (1)

$$J_p = \frac{D_p \varepsilon}{h_p} C_d \quad \text{----------(1)}$$

Where, D_p is diffusion coefficient of the permeate in the intercellular spaces, h_p is the path length of the paracellular route, ε is the area fraction of the paracellular route and C_d is the donor drug concentration. Similarly, flux of drug through the membrane under sink condition for transcellular route can be written as Eq. (2).

$$J_c = \frac{(1-\varepsilon) D_c K_c}{h_c} C_d \quad \text{----------(2)}$$

Where, K_c is partition coefficient between lipophilic cell mem- brane and the aqueous phase, D_c is the diffusion coefficient of the drug in the transcellular spaces and h_c is the path length of the transcellular route [14].

In very few cases absorption also takes place by the process of endocytosis where the drug molecules were engulfed by the cells. It is unlikely that active transport processes operate within the oral mucosa; however, it is believed that acidic

stimulation of the salivary glands, with the accompanying vasodilatation, faci- litates absorption and uptake into the circulatory system[15].

The absorption potential of the buccal mucosa is influenced by the lipid solubility and molecular weight of the diffusant. Absorption of some drugs via the buccal mucosa is found to increase when carrier pH is lowered and decreased with an increase of pH [16] . However, the pH dependency that is evident in absorption of ionizable compounds reflects their partitioning into the epithelial cell membrane, so it is likely that such compounds will tend to penetrate transcellularly [17]. Weak acids and weak bases are subjected to pH-dependent ionization. It is presumed that ionized species penetrate poorly through the oral mucosa compared with non-ionized species. An increase in the amount of non-ionized drug is likely to increase the permeability of the drug across an epithelial barrier, and this may be achieved by a change of pH of the drug delivery system. It has been reported that pH has effect on the buccal permeation of drug through oral mucosa [18]. The diffusion of drugs across buccal mucosa was not related to their degree of ionization as calculated from the Henderson–Hasselbalch equation and thus it is not helpful in the prediction of membrane diffusion of weak acidic and basic drugs [19] .

In general, for peptide drugs, permeation across the buccal epithelium is thought to be through paracellular route by passive diffusion. Recently, it was reported that drugs that have a mono- carboxylic acid residue could be delivered into systemic circulation from the oral mucosa via its carrier [20] . The permeability of oral mucosa and the efficacy of penetration enhancers have been inves- tigated in numerous *in vivo* and *in vitro* models. Various kinds of diffusion cells, including continuous flow perfusion chambers, Ussing chambers, Franz cells and Grass–Sweetana, have been used to determine the permeability of oral mucosa [21]. Cultured epi- thelial cell lines have also been developed as an *in vitro* model for studying drug transport and metabolism at biological barriers as well as to elucidate the possible mechanisms of action of pene- tration enhancers [22-23]. Recently, TR146 cell culture model was suggested as a valuable *in vitro* model of human buccal mucosa for permeability and metabolism studies with enzymatically labile drugs, such as leu-enkefalin, intended for buccal drug delivery [24] .

Barriers to penetration across buccal mucosa

The barriers such as saliva, mucus, membrane coating granules, basement membrane etc retard the rate and extent of drug absorption through the buccal mucosa. The main penetration barrier exists in the outermost quarter to one third of the epithelium [8].

Membrane coating granules or cored granules

In nonkeratinized epithelia, the accumulation of lipids and cytokeratins in the keratinocytes is less evident and the change in morphology is far less marked than in keratinized epithelia. The mature cells in the outer portion of nonkeratinized epithelia become large and flat retain nuclei and other organelles and the cytokeratins do not aggregate to form bundles of filaments as seen in keratinizing epithelia. As cells reach the upper third to quarter of the epithelium, membrane-coating granules become evident at the superficial aspect of the cells and appear to fuse with the plasma membrane so as to extrude their contents into the intercellular space. The membrane-coating granules found in nonkeratinizing epithelia are spherical in shape, membrane-bounded and measure about 0.2 μm in diameter [25]. Such granules have been observed in a variety of other human nonkeratinized epithelia, including uterine cervix [26] and esophagus [27]. However, current studies employing ruthenium tetroxide as a post-fixative indicate that in addition to cored granules, a small proportion of the granules in nonkeratinized epithelium do contain lamellae, which may be the source of short stacks of lamellar lipid scattered throughout the intercellular spaces in the outer portion of the epithelium. In contrast to the intercellular spaces of stratum corneum, those of the superficial layer of nonkeratinizing epithelia contain electron lucent material, which may represent nonlamellar phase lipid, with only occasional short stacks of lipid lamellae.

Basement membrane

Although the superficial layers of the oral epithelium represent the primary barrier to the entry of substances from the exterior, it is evident that the basement membrane also plays a role in limiting the passage of materials across the junction between epithelium and connective tissue. A similar mechanism appears to operate in the opposite direction. The charge on the constituents of the basal lamina may limit the rate of penetration of lipophilic compounds that can traverse the superficial epithelial barrier relatively easily [28].

Mucus

The epithelial cells of buccal mucosa are surrounded by the intercellular ground substance called mucus with the thickness varies from 40 μm to 300 μm[29]. Though the sublingual glands and minor salivary glands contribute only about 10% of all saliva, together they produce the majority of mucus and are critical in maintaining the mucin layer over the oral mucosa[30]. It serves as an effective delivery vehicle by acting as a lubricant allowing cells to move relative to one another and is believed to play a major role in adhesion of mucoadhesive drug delivery systems [31]. At buccal pH, mucus can form a strongly cohesive gel structure that binds to the epithelial cell surface as a gelatinous layer [8] .Mucus mole- cules are able to join together to make polymers or an extended three-dimensional network. Different types of mucus are produced, for example G, L, S, P and F mucus, which form different network of gels. Other substances such as ions, protein chains, and enzymes are also able to modify the interaction of the mucus molecules and, as a consequence, their biophysical properties [32].

Mucus is composed chiefly of mucins and inorganic salts suspended in water. Mucins are a family of large, heavily glycosylated proteins composed of oligosaccharide chains attached to a protein core. Three quarters of the protein core are heavily glycosylated and impart a gel like characteristic to mucus. Mucins contain approximately 70–80% carbohydrate, 12–25% protein and up to 5% ester sulphate [33]. The dense sugar coating of mucins gives them considerable water-holding capacity and also makes them resistant to proteolysis, which may be important in maintaining mucosal barriers [4].

Mucins are secreted as massive aggregates by prostaglandins with molecular masses of roughly 1 to 10 million Da. Within these aggregates, monomers are linked to one another mostly by non- covalent interactions, although intermolecular disulphide bonds also play a role in this process. Oligosaccharide side chains con- tain an average of about 8–10 monosaccharide residues of five different types namely L-fucose, D-galactose, *N*-acetyl-D-glucos- amine, *N*-acetyl-D-galactosamine and sialic acid. Amino acids present are serine, threonine and proline [34]. Because of the presence of sialic acids and ester sulfates, mucus is negatively charged at physiological salivary pH of 5.8–7.4 [8].

At least 19 human mucin genes have been distinguished by cDNA cloning-MUC1, 2, 3A, 3B, 4,5AC, 5B, 6–9, 11–13, and 15–19. Mucin genes encode mucin monomers that are synthesized as rod-shaped apomucin covers that are post translationally modified by exceptionally abundant glycosylation. Two distinctly dif-

ferent regions are found in mature genes. The amino- and carboxy- terminal regions are very lightly glycosylated, but rich in cysteines, which are likely, involved in establishing disulfide linkages within and among mucin monomers. A large central region formed of multiple tandems repeats of 10 to 80 residue sequences in which up to half of the amino acids are serine or threonine. This area becomes saturated with hundreds of O-linked oligosaccharides also found on mucins, but much less abundantly [4].

Mucins are characterized not only by large molecular masses but also by large molecular mass distributions, as seen by analytical ultra-centrifugation, and by the powerful technique of size exclusion chromatography coupled to multi-angle laser light sca- ttering[35-36]. In solution, mucins adopt a random-coil conformation occupying a time averaged spheroidal domain as shown by hydrodynamics and critical-point-drying[37]. Electron mi- croscopy. Mucins, which are different, are the submaxillary mu- cins, with a lower carbohydrate content and different structure [38].

Saliva

The mucosal surface has a salivary coating estimated to be 70 µm thick[39], which act as unstirred layer. Within the saliva there is a high molecular weight mucin named MG1 [40] that can bind to the surface of the oral mucosa so as to maintain hydration, provide lubrication, concentrate protective molecules such as secretory immunoglobulins, and limit the attachment of micro-organisms. Several independent lines of evidence suggest that saliva and salivary mucin contribute to the barrier properties of oral mucosa [41]. The major salivary glands consist of lobules of cells that secrete saliva; parotids through salivary ducts near the upper teeth, submandibular under the tongue, and the sublingual through many ducts in the floor of the mouth. Besides these glands, there are 600–1000 tiny glands called minor salivary glands located in the lips, inner cheek area (buccal mucosa), and extensively in other linings of the mouth and throat [42]. Total output from the major and minor salivary glands is termed as whole saliva, which at normal conditions has flow rate of 1–2 ml/min[43]. Greater salivary output avoids potential harm to acid-sensitive tooth enamel by bathing the mouth in copious neutralizing fluid [44]. With stimulation of salivary secretion, oxygen is consumed and vasodilator substances are produced; and the glandular blood flow increases, due to increased glandular metabolism [45]. Saliva is composed of 99.5% water in addition to proteins, glycoproteins and electrolytes. It is high in potassium (7 × plasma), bicarbonate (3 × plasma), calcium, phosphorous, chloride, thiocyanate and urea and low in sodium (1/10 × plasma). The normal pH of saliva is 5.6-7. Saliva contains enzymes namely α-amylase (breaks 1–4 glycosidic bonds), lysozyme (protective, digests bacterial cell walls) and lingual lipase (break down the fats) [46].

Saliva serves multiple important functions. It moistens the mouth, initiates digestion and protects the teeth from decay. It also controls bacterial flora of the oral cavity. Because saliva is high in calcium and phosphate, it plays a role in mineralization of new teeth repair and precarious enamel lesions. It protects the teeth by forming "protective pellicle". This signifies a saliva protein coat on the teeth, which contains antibacterial compounds. Thus, problems with the salivary glands generally result in rampant dental caries. Lysozyme, secretory IgA, and salivary peroxidase play important roles in saliva's antibacterial actions. Lysozyme agglutinates bacteria and activates autolysins. Ig A interferes with the adherence of microorganisms to host tissue. Peroxidase breaks down salivary thiocyanate, which in turn, oxidizes the enzymes involved in bacterial glycolysis. However, salivary flow rate may play role in oral hygiene. Intraoral complications of salivary hypofunction may cause candidiasis, oral lichen planus, burning mouth syndrome, recurrent aphthous ulcers and dental caries. A constant flowing down of saliva within the oral cavity makes it very difficult for drugs to be retained for a significant amount of time in order to facilitate absorption in this site [44]. The other important factor of great concern is the role of saliva in development of dental caries. Salivary enzymes act on natural polysaccharidic polymers that hasten the growth of mutants of streptococci and other plaque bacteria leading to development of dental caries.

In general, intercellular spaces pose as the major barrier to permeation of lipophilic compounds, and the cell membrane which is lipophilic in nature acts as the major transport barrier for hydrophilic compounds because it is difficult to permeate through the cell membrane due to a low partition coefficient [45]. Permeability between different regions of the oral cavity vary greatly because of the diverse structures and functions. In general, the permeability is based on the relative thickness and degree of keratinization of these tissues in the order of sublingual> buccal> palatal. The permeability of the buccal mucosa was estimated to be 4–4000 times greater than that of the skin[46].

II. FORMULATION DESIGN

Buccal adhesive drug delivery systems with the size 1–3 cm^2 and a daily dose of 25 mg or less are preferable. The maximal duration of buccal delivery is approximately 4–6 h [47].

Pharmaceutical considerations

Great care needs to be exercised while developing a safe and effective buccal adhesive drug delivery device. Factors influencing drug release and penetration through buccal mucosa, organoleptic factors, and effects of additives used to improve drug release pattern and absorption, the effects of local drug irritation caused at the site of application are to be considered while designing a formulation[48].

Buccal adhesive polymers

Polymer is a generic term used to describe a very long molecule consisting of structural units and repeating units connected by covalent chemical bonds. The term is derived from the Greek words: *polys* meaning *many*, and *meros* meaning *parts*[49]. The key feature that distinguishes polymers from other molecules is the repetition of many identical, similar, or complementary molecular subunits in these chains. These subunits, the monomers, are small molecules of low to moderate molecular weight, and are linked to each other during a chemical reaction called polymerization. Instead of being identical, similar monomers can have varying chemical substituents. The differences between monomers can affect properties such as solubility, flexibility, and strength. The term buccal adhesive polymer covers a large, diverse group of molecules, including substances from natural origin to biodegradable grafted co-polymers and thiolated polymers[50].

Bioadhesive formulations use polymers as the adhesive component. These formulations are often water soluble and when in a dry form attract water from the biological surface and this water transfer leads to a strong interaction. These polymers also form viscous liquids when hydrated with water that increases their retention time over mucosal surfaces and may lead to adhesive interactions. Bioadhesive polymers should possess certain physicochemical features including hydrophilicity, numerous hydrogen bond-forming groups, flexibility for interpenetration with mucus and epithelial tissue, and viscoelastic properties[51].

Ideal characteristics [52]

Should demonstrate acceptable shelf life.

Should have optimum molecular weight.

Should possess adhesively active groups.

Should have required spatial conformation.

Should be sufficiently cross-linked but not to the degree of suppression of bond forming groups.

Should not aid in development of secondary infections such as dental caries.

Polymer and its degradation products should be non-toxic, non-irritant and free from leachable impurities.

Should have good spreadability, wetting, swelling and solubility and biodegradability properties.

pH should be biocompatible and should possess good viscoelastic properties.

Should adhere quickly to buccal mucosa and should possess sufficient mechanical strength.

Should possess peel, tensile and shear strengths at the bioadhesive range.

Polymer must be easily available and its cost should not be high.

Should show bioadhesive properties in both dry and liquid state.

Should demonstrate local enzymeinhibition and penetration enhancement properties.

Some representative polymers

Hydrogels. Hydrogels, often called as "wet" adhesives because they require moisture to exhibit the adhesive property. They are usually considered to be cross linked water swollen polymers having water content ranging from 30% to 40% depending on the polymer used. These are hydrophilic matrices that absorb water when placed in an aqueous media. This may be supplied by the saliva, which may also act as the dissolution medium. They are structured in such a manner that the crosslinking fibers present in their matrix effectively prevent them from being dissolved and thus help them in retaining water. When drugs are loaded into these hydrogels, as water is absorbed into the matrix, chain relaxation occurs and drug molecules are released through the spaces or channels within the hydrogel network. Polymers such as polyacrylates (carbopol and polycarbophil), ethylene vinyl alcohol, polyethylene oxide, poly vinyl alcohol, poly (*N*-acryloylpyrrolidine), polyoxyethylenes, self-cross linked gelatin, sodium alginate, natural gums like guar gum, karaya gum, xanthan gum, locust bean gum and cellulose ethers like methyl cellulose, hydroxypropyl cellulose, hydroxyl propyl methyl cellulose, sodium carboxy methyl cellulose etc. form part of the family of hydrogels [53].

Copolymers

Researchers are currently working on carrier systems containing block copolymers rather than using single polymeric system. Copolymerization with two or more different monomers results in chains with varied properties. A block copolymer is formed when the reaction is carried out in a stepwise manner, leading to a structure with long sequences or blocks of one monomer alternating with long sequences of the other. These networks when composed of hydrophilic and hydrophobic monomers are called polymer micelle. These micelles are suitable for enclosing individual drug molecules. Their hydrophilic outer shells help to protect the cores and their contents from chemical attack by aqueous medium. Most micelle-based systems are formed from poly (ethylene oxide)-*b*-polypropylene-*b*-poly (ethylene oxide) tri- block network. There are also graft copolymers, in which entire chains of one kind (e.g., polystyrene) are made to grow out of the sides of chains of another kind (e.g., polybutadiene), resulting in a product that is less brittle and more impact-resistant. Thus, block and graft copolymers can combine the useful properties of both constituents and often behave as quasi-two-phase systems [54].

Multifunctional polymers

These are the bioadhesive polymers having multiple functions. In addition to the possession of bioadhesive properties, these polymers will also serve several other functions such as enzyme inhibition, permeation enhancing effect etc. Examples are polyacrylates, po- lycarbophil, chitosan etc.[55].

Thiolated polymers

These are the special class of multifunctional polymers also called thiomers. These are hydrophilic macromolecules exhibiting free thiol groups on the polymeric backbone. Due to these functional groups various features of well-established polymeric excipients such as poly (acrylic acid) and chitosan were strongly improved. Thiolated polymers designated thiomers are capable of forming disulphide bonds with cysteine-rich subdomains of mucus glycoproteins covering mucosal membranes. Consequently, the bridging structure most commonly used in biological systems is utilized to bind drug delivery systems on the mucosal membranes. By immobilization of thiol groups the mucoadhesive properties of poly (acrylicacid) and chitosan, wasimprovedto100-foldto250- fold [56].

Thiomers are capable of forming intra- and inter-chain disulphide bonds within the polymeric network leading to strongly improved cohesive properties and stability of drug delivery systems such as matrix tablets. Due to the formation of strong covalent bonds with mucus glycoproteins, thiomers show the strongest mucoadhesive properties of all so far tested polymeric excipients via thioldisulphide exchange reaction and an oxidation process. Zinc dependent proteases such as aminopeptidases and carboxy peptidases are inhibited by thiomers. The underlying mechanism is based on the capability of thiomers to bind zinc ions and this property is highly beneficial for oral administration of protein and peptide drugs. They also exhibit permeation-enhancing effects for the paracellular uptake of drugs based on a glutathione-mediated opening process of the tight junctions [57].

Milk protein

A particular example is a milk protein concentrate containing a minimum of 85% of proteins such as Prosobel L85, LR85F at concentration of 15% to 50%, preferably 20% to 30% in a bioadhesive tablet showed good bioadhesive property [58].

In general

Cationic and anionic polymers bind more effectively than neutral polymers, Anionic polymers with sulphate groups bind more effectively than those with carboxylic groups, Polyanions are better than polycations in terms of binding potential and toxicity, Water-insoluble polymers give greater flexibility in dosage form design compared to rapidly or slowly dissolving water-soluble polymers, Degree of binding is proportional to the charge density on the polymer [59].

Factors governing drug release from a polymer

For a given drug the release kinetics from the polymer matrix could be governed predominantly by the polymer

morphology and excipients present in the system. Drug release from a polymeric material takes place either by the diffusion or by polymer degradation or by a combination of the both. Polymer degradation generally takes place by the enzymes or hydrolysis either in the form of bulk erosion or surface erosion [60].

Polymer morphology

The polymer matrix could be formulated as macro or nanospheres, gel film or an extruded shape (cylinder, rod etc). Also the shape of the extruded polymer can be important to the drug release kinetics. It has been shown that zero order release kinetics can be achieved using hemispherical polymer form [61].

Excipients

The main objective of incorporating excipients in the polymer matrix is to modulate polymer degra- dation kinetics. Studies carried out have shown that by in- corporating basic salts as excipients slow down the degradation and increases the stability of protein polymers. Similarly hydro- philic excipients can accelerate the release of drugs although they may also increase the initial burst effect [62].

Physiological considerations

Physiological considerations such as texture of buccal mucosa, thickness of the mucus layer, its turn over time, effect of saliva and other environmental factors are to be considered in designing the dosage forms. Saliva contains moderate levels of esterases, carbohydrases, and phosphatases that may degrade certain drugs. Although saliva secretion facilitates the dissolution of drug, involuntary swallowing of saliva also affects its bioavailability. Hence development of unidirectional release systems with backing layer results high drug bioavail- ability [63].

Pharmacological considerations

Drug absorption depends on the partition coefficient of the drugs. Generally lipophilic drugs absorb through the transcellular route, whereas hydrophilic drugs absorb through the paracellular route. Chemical modification may increase drug penetration through buccal mucosa. Increasing nonionized fraction of ionizable drugs increases drug penetration through transcellular route. In weakly basic drugs, the decrease in pH increases the ionic fraction of drug but decreases its permeability through buccal mucosa. Electrostatic interactions of drugs such as tetracycline, hydrogen bonding with drugs like urea and hydrophobic interactions with drugs like testosterone with mucin will decrease rate of absorption. Residence time and local concentration of the drug in the mucosa, the amount of drug transported across the mucosa into the blood are the responsible factors for local or systemic drug delivery. Optimization by a suitable formula- tion design hastens drug release from the dosage form and taken up by the oral mucosa. Drugs such as buprenorphine, testosterone, fentanyl, nifedipine and several peptides such as insulin, thyrotropin-releasing hormone, and oxytocin have been tried to deliver via the buccal route. However the relative bioavailabilities of peptides by the buccal route were still low due to its poor permeation and enzymatic barrier of buccal mucosa but can be improved by the incorporation of penetration enhancers and/or enzyme inhibitors. Previous drug absorption studies have demonstrated that oral mucosal absorption of amines and acids at constant concentration are proportional to their partition coefficients. Similar dependencies on partition coefficients were obtained from acyclovir, β- adrenoreceptor blocking agents, substituted acetanilide, and others [64].

Permeation enhancers

Membrane permeation is the limiting factor for many drugs in the development of buccal adhesive delivery devices. The epithelium that lines the buccal mucosa is a very effective barrier to the absorption of drugs. Substances that facilitate the permeation through buccal mucosa are referred as permeation enhancers. As most of the penetration enhancers were originally designed for purposes other than absorption enhancement, a systemic search for safe and effective penetration enhancers must be a priority in drug delivery. The goal of designing penetration enhancers, with improved efficacy and reduced toxicity profile is possible by under- standing the relationship between enhancer structure and the effect induced in the membrane and of course, the mechanism of action. However, the selection of enhancer and its efficacy depends on the physicochemical properties of the drug, site of administration, nature of the vehicle and other excipients. In some cases usage of enhancers in combination has shown synergistic effect than the individual enhancers. The efficacy of enhancer in one site is not same in the other site because of differences in cellular morphology, membrane thickness, enzymatic activity, lipid composition and potential protein interactions are structural and functional properties. Penetration enhancement to the buccal membrane is drug specific. Effective penetration enhancers for transdermal or intestinal drug delivery may not have similar effects on buccal drug delivery because of structural differences; however, enhancers used to improve drug permeation in other absorptive mucosae improve drug penetration through buccal mucosa. These permeation enhancers should be safe and non-toxic, pharma- cologically and chemically inert, non-irritant, and nonaller- genic. However, examination of penetration route for transbuccal delivery is important because it is fundamental to select the proper penetration enhancer to improve the drug permeability. The different permeation enhancers available are [65].

Chelators: EDTA, citric acid, sodium salicylate, methoxy salicylates.

Surfactants: sodium lauryl sulphate, polyoxyethylene, Polyoxyethylene-9-laurylether, Polyoxythylene-20-cety- lether, Benzalkonium chloride, 23-lauryl ether, cetylpyr- idinium chloride, cetyltrimethyl ammonium bromide.

Bile salts: sodium glycocholate, sodium deoxycholate, sodium taurocholate, sodium glycodeoxycholate, sodium taurodeoxycholate.

Fatty acids: oleic acid, capric acid, lauric acid, lauric acid/ propylene glycol, methyloleate, lysophosphatidylcholine, phosphatidylcholine.

Non-surfactants: unsaturated cyclic ureas.

Inclusion complexes: cyclodextrins.

Others: aprotinin, azone, cyclodextrin, dextran sulfate, menthol, polysorbate 80, sulfoxides and various alkyl glycosides.

Thiolated polymers: chitosan-4-thiobutylamide, chitosan- 4-thiobutylamide/GSH, chitosan-cysteine, Poly (acrylic acid)-homocysteine, polycarbophil-cysteine, polycarbo- phil-cysteine/GSH, chitosan-4-thioethylamide/GSH, chit- osan-4-thioglycholic acid.

Mechanisms of action

Mechanisms by which penetration enhancers are thought to improve mucosal absorption are as follows [66]

Changing mucus rheology: Mucus forms viscoelastic layer of varying thickness that affects drug absorption. Further, saliva covering the mucus layers also hinders the absorption. Some permeation enhancers' act by reducing the viscosity of the mucus and saliva overcomes this barrier.

Increasing the fluidity of lipid bilayer membrane: The most accepted mechanism of drug absorption through buccal mucosa is intracellular route. Some enhancers disturb the intracellular lipid packing by interaction with either lipid packing by interaction with either lipid or protein components.

Acting on the components at tight junctions: Some enhancers act on desmosomes, a major component at the tight junctions there by increases drug absorption. *By overcoming the enzymatic barrier*: These act by inhibiting the various peptidases and proteases present within buccal mucosa, thereby overcoming the enzymatic barrier. In addition, changes in membrane fluidity also alter the enzymatic activity indirectly.

Increasing the thermodynamic activity of drugs: Some enhancers increase the solubility of drug there by alters the partition coefficient. This leads to increased thermo-dynamic activity resulting better absorption.

Surfactants such as anionic, cationic, nonionic and bile salts increases permeability of drugs by perturbation of intercellular lipids whereas chelators act by interfering with the calcium ions, fatty acids by increasing fluidity of phospholipids and positively charged polymers by ionic interaction with negative charge on the mucosal surface. Chitosan exhibits several favorable properties such as biodegradability, biocompatibility and antifungal/antimicrobial properties in addition to its potential bioadhesion and absorption enhancer.

III. MUCO/BIOADHESION

Bioadhesion is the phenomenon between two materials, which are held together for extended periods of time by interfacial forces. It is generally referred as bioadhesion when interaction occurs between polymer and epithelial surface; mucoadhesion when occurs with the mucus layer covering a tissue. Generally bioadhesion is deeper than the mucoadhesion. However, these two terms seem to be used interchangeably. It is interesting that the interaction between the layers adsorbed from whole saliva resembles the one previously reported between layers of adsorbed gastric mucins, which points to a strong contribution to the interaction of high molecular weight glycoproteins [67].

Bio/mucoadhesive forces

The common nature of all adhesive events, interfacial phenomena and forces that are involved in bioadhesion are strongly related to those considered in classical colloid and surface science. Intermolecular forces are electromagnetic forces which act between molecules or between widely separated regions of a macromolecule. These are fundamentally electrostatic interactions or electrodynamic interactions. Such forces may be either attractive or repulsive in nature. They are conveniently divided into two classes: short-range forces, which operate when the centers of the molecules are separated by 3 angstroms or less and long-range forces, which operate at greater distances. Generally, if molecules do not tend to interact chemically, the short-range forces between them are repulsive. These forces arise from interactions of the electrons associated with the molecules and are also known as exchange forces. Molecules that interact chemically have attractive exchange forces; these are also known as valence forces. Mechanical rigidity of molecules and effects such as limited compressibility of matter arise from repulsive exchange forces. Long-range forces, or van der Waal's forces as they are also called, are attractive and account for a wide range of physical phenomena, such as friction, surface tension, adhesion and cohesion of liquids and solids, viscosity, and the discrepancies between the actual behavior of gases and that predicted by the ideal gas law [68].

Many theories have been proposed to explain the forces that underpin bioadhesion. They are: Electronic theory, Adsorption theory, Wetting theory, Diffusion theory and Fracture theory, etc.[69].

However, there is yet to be a clear explanation. As bioadhesion occurs between inherently different mucosal surfaces and formulations that are solid, semisolid and liquid, it is unlikely that a single, universal theory will account for all types of adhesion observed. In biological systems it must be recognized that, owing to the amphiphilicity of many biological macromolecules, orientation effects can often occur at inter- faces. These are crucially important and have in fact been reported to be so dramatic as to change overall long-range interactions from being purely repulsive to their becoming attractive. For any type of charged surface, such as biosurfaces, it is common to distinguish between pure electrostatic repulsive forces, which oppose adhesion, and attractive forces, which, if the surfaces come close enough, will strive to bring the interacting bodies together. This balanced relationship between repulsive and attractive interactions is expressed in the DLVO theory. In biological systems, interactions can be more complex, as they often take place in high ionic strength aqueous media and in the presence of macromolecules. Therefore electrostatic contributions may be less important, at least at long range, in favor of force components such as steric forces, hydrophobic interactions, and hydration forces [70].

Vander Waal's forces

The attractive forces included in the DLVO theory are normally termed van der Waal's forces and will arise in a number of ways. These may be further divided into the following three components [71]:

I. London dispersion forces: These are also called as dispersion forces. These originate out of the electronic motions in paired molecules and give rise to attractive interactions. These forces involve the attraction between temporarily induced dipoles in nonpolar molecules (often disappear within a second). This polarization can be induced either by a polar molecule or by the repulsion of negatively charged electron clouds in nonpolar molecules. These results when two atoms belonging to different molecules are brought sufficiently close together. These interactions involve a force of about 0.5–1 K cal/mole. London Dispersion forces exist between all atoms.

II. Dipole–dipole interactions: These are also called Keesom interactions after Willem Hendrik Keesom who produced the first mathematical description in 1921, are the forces that occur between two molecules with permanent dipoles. These work in a similar manner to ionic inter- actions, but are weaker because only partial charges are involved. These are due to attraction between polar groups. These have force of 1–7 K cal/mole. Dipole– dipole interactions also come from partial charges another order of magnitude weaker.

III. Debye type forces: These are the interactions between permanent and induced dipoles. Permanent dipoles can induce a transient electric dipole in non-polar molecules and produce dipole induced dipole interactions. These interactions involve a force of about 1–3 K cal/mole.

The non-retarded van der Waal's force is inversely proportional to the square of the distance between two spherical particles, where the proportionality constant is the Hamaker constant, which has the dimension of energy, can be used to describe the strength of the van der Waal's interaction and is dependent on the properties of the involved particles and on the medium where the interaction takes place [72].

Hydrogen bonding

Hydrogen bonding is basically an electrostatic interaction that arises when a hydrogen atom bound to an electronegative atom, e.g., nitrogen, oxygen, or fluorine, interacts with another electronegative atom. The result is a dipolar molecule. The hydrogen atom has a partial positive charge and hence can interact with another highly electronegative atom in an adjacent molecule. This results in a stabilizing interaction that binds the two molecules together. The force is short range and highly directional. In a more hydrophobic environment, hydrogen bonds become significant and are essential in the formation of stable structures. Bond energy serves as a measure of strength of bonds. Magnitude of bond energy for hydrogen bond is between 10 and 20 kJ/mol. Role of hydrogen bonding in interaction between mucoadhesive and mucin at gastric pH was studied by Tobyn et al. The bonding is stronger and is directional. The directional nature of hydrogen bonding requires the two molecules to adopt a specific relative geometry [73].

Disulphide bridging

A disulfide bond (SS-bond), also called a disulfide bridge, is a strong covalent bond between two sulfhydryl (–SH) groups. Oxidation of the thiol group yields a disulfide (S–S) bond. This bond is very important to the folding, structure, and function of proteins. Due to the formation of strong covalent bonds with mucus glycoproteins, thiomers show the strongest mucoadhesive properties of all so far tested polymeric excipients via thioldisulphide exchange reaction and an oxidation process as shown in Figure 2 [74].

Hydration forces

A type of short-range (< 1 nm) repulsive interaction, suggested as originating from the binding of water molecules to polar surface sites, has been observed between phospholipids and solid surfaces under certain conditions. This hydration force is believed to be particularly important in biological systems, since it prevents contact even in the absence of charge–charge repulsion [75].

Electrostatic double-layer forces

A charged surface is always surrounded by a cloud of counter ions (double-layer), which balances the surface charge. When two surfaces with the same charge approach each other, a repulsive force will arise due to the overlap of the double layers. This is the origin of the electrostatic double-layer forces, which can be described by the so-called Poisson–Bolzmann equation. These forces decay exponentially with the surface separation, with a decay length that decreases with increasing ionic strength in the surrounding medium. It should be noted that specific dispersion force-induced ion adsorption could some- times dominate at charged interfaces, thereby making it virtually impossible to distinguish between the contributions of electrostatic and dispersion forces. In biological fluids, which generally carry a large net negative charge, contribute significantly to the decay length already at low concentrations. Thus, the decay length in saliva is likely to be less than the value of approximately 1.0 nm calculated from its salt composition. Any increase in ionic strength, increases adhesion to negatively charged surfaces; this was assigned to less repulsion between the surface and the adhering cells [76].

Hydrophobic interactions

Hydrophobic effect is another particularly important phenomenon with respect to bioadhesion related to the presence of water. It is the property that nonpolar molecules like to self-associate in the presence of aqueous solution. It has been assigned to the tendency of water molecules to form ordered structures in proximity to non-polar molecular domains and may give rise to attractive interactions between non-polar residues such as hydrocarbon side chains. The hydrophobic effect is usually described in the context of protein folding, protein–protein interactions, nucleic acid structure, and protein–small molecule interactions. In the case of protein folding, it is used to explain why many proteins have a hydrophobic core which consists of hydrophobic amino acids, such as alanine, valine, leucine, isoleucine, phenylalanine, and methionine grouped together; often coiled-coil structures form around a central hydrophobic axis. The energetics of DNA tertiary structure assembly were determined by Eric Kool to be mostly caused by the hydrophobic effect, as opposed to Watson–Crick base pairing [77].

The hydrophobic effect can be nullified to a certain extent by lowering the temperature of the solution to near zero degrees; at such temperatures, water prefers to be in an ordered structure and the order generated by hydrophobic patches is no longer as energetically unfavorable. This is neatly demonstrated by the increased solubility of benzene in water at temperatures lower than room temperature. On the macroscopic level, long-range attractive forces have been observed between hydrophobic surfaces formed by adsorption or deposition of amphiphilic molecules and are believed to be non-equilibrium forces [78].

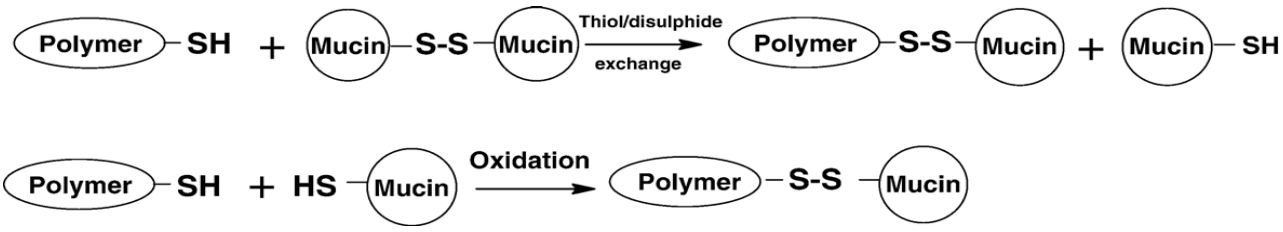

Figure 2 Formation of covalent bonds between thiolated polymers and mucin glycoproteins

It should be noted that the origin of the long-range attractive forces between hydrophobic surfaces is controversial, but their occurrence has been related to instability of the deposited monolayer. Strength of these interactions is about 0.37 kcal/mol [79].

Steric forces

Repulsive steric interactions or steric forces appear as the result of the increasing concentration of molecular segments that occurs when surfaces bearing for example bound macro-molecules come close to each other and therefore considered to be important in biological systems. The maximum possible number of molecular contacts between an adhesive and its substrate may be greatly restricted by the steric aspects of molecular geometry [80].

Covalent bonds

Like metallic bonds, covalent bonds are characterized by the electrons that are shared between the engaged atoms. Covalent bonds operate only over short interatomic distances (1– 2× 10^{-1} nm). They tend to decrease in strength with increasing bond-length, and are oriented at well-defined angles. Unless chemical reactions take place, based on the formation or breakup of for example disulphide bridges, covalent bonds are unlikely to be important in bioadhesion processes under physiological conditions. On the basis of molecular interactions, the interaction between two molecules is composed of attraction and repulsion. Attractive interactions arise from weak forces such as van der Waal's forces, electrostatic attraction, hydrogen bonding, hydrophobic interac- tions and/or strong forces, which are covalent in nature. Repulsive interactions occur because of electrostatic and steric repulsion. For muco/bioadhesion to occur, the attractive interactions should be larger than nonspecific repulsion [81].

Steps involved in the process of bio/mucoadhesion are

i.Spreading, wetting, swelling and dissolution of bio/ mucoadhesive polymer at the interface, initiates intimate molecular contact at the interface between the polymer and the epithelial/mucus layer.

Ii. Interdiffusion and interpenetration between the chains of the adhesive polymer and the mucus/epithelial surface resulting physical cross links or mechanical interlocking.

Iii. Adsorption: The orientation of the polymers at the interface so that adhesive bonding across the interface is possible and

Iv. Formation of secondary chemical bonds between the polymer chains and mucin molecules [82].

Methods for measuring mucoadhesion

These tests are important during the design and development of a mucoadhesive release system to study compatibility, stability, surface analysis and bioadhesive bond strength. These tests are broadly classified in to qualitative methods and quantitative methods [83].

Quantitative methods

These are also called macroscopic methods. The majority of the quantitative bio and/or mucoadhesion measurement methods found in the literature are based on measuring the force required to break the adhesive bond between the model membrane and the adhesive. Depending on the direction in which the adhesive is being separated from the substrate, peel, shear, and tensile forces can be measured [83].

Determination of peel strength

The peel adhesion tests are mainly used for buccal and transdermal patches. 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 Figure 3. Fracture Energy (G)

$$G = \frac{P(1-Cos\ \theta)}{w} = W\$(1+k) \quad \text{----------(3)}$$

Where P is the peel force;w is the peel width; $W°$ is the intrinsic work of adhesion and k is the proportionality constant that accounts for hysteretic losses. Peel work is the sum of the following components [84]

Surface energy that results from the creation of two free surfaces (energy of dewetting) also referred to as the intrinsic work of adhesion (or cohesion)

Bulk energy that dissipates into the stripping member

Strain energy in the newly detached strip

Intrinsic work of adhesion (or cohesion) is independent of the following:

Peel rate (speed), Peel angle, Thickness of the adhesive and Thickness of the stripping member

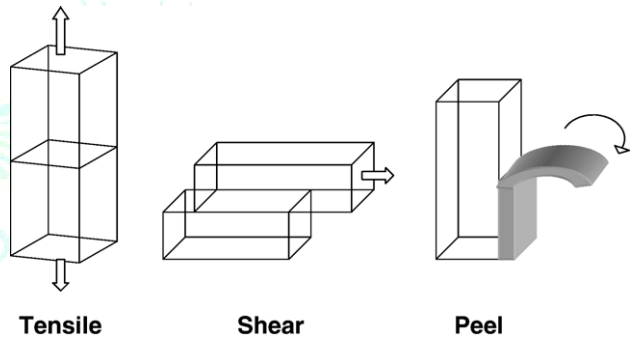

Figure 3. Representation of peel, shear and tensile forces

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 [85]: s ¼ F=A

Where, τ shear stress, force *A and* 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 centimeter of the surface of the upper plate to keep it in motion, the shear stress in the fluid is 1 dyn/cm^2 at any point between the two plates. Shear stress measures the force that requires causing the bioadhesive to slide with respect to the mucus layer in a direction parallel to their plane of contact as shown in Figure 3.

Sam et al. studied the mucoadhesiveness of Ca polycarbophil, sodium CMC, HPMC using homogenized mucus from pig in-testine as model substrate by modified wilhelmy plate surface tension apparatus. Similarly, Smart et al. studied mucoadhesive strength of CP 934, Na CMC, HPMC, gelatin, PVP, acacia, PEG, pectin, tragacanth and sodium alginate was measured by the force required to pull the plate out of the solution is determined under constant experimental conditions by using mucus from guinea pig intestine as model substrate by Wilhelmy plate method, where a glass plate

suspended from a microbalance, which was dipped in a temperature-controlled mucus sample. Instead of biological substrates, Ishida et al. used glass plates as model substrate by shearing stickiness apparatus and Gurney et al. used polymethyl methacrylate to study shear stress of carbapol and sodium CMC by Instron model 1114, respectively [86].

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[87].

There are three typical definitions of tensile strength[88]:

Yield Strength- The stress a material can withstand without permanent deformation.

Ultimate Strength- The maximum stress a material can withstand.

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 polymers.

Lehr et al. 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 2 min, 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. A shear stress was applied by lowering the plates and model mucosa at a rate of 2 mm min^{-1} until adhesive joint failure occurred (Figure 4).

Many researchers studied shear strength of polymers such as polyacrylic acid, hydroxy propylcellulose, carbapol 934, HPMC etc. using buccal mucosa as substrate by using different instruments such as tensile tester, modified pan balance etc [89].

Colloidal gold staining method

Park proposed the colloidal gold staining technique for the study of bioadhesion. The technique 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[90].

Direct staining method

It is a novel technique to evaluate polymer adhesion to human buccal cells following exposure to aqueous polymer dispersion, 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 0.25 M 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 dis- persions of these polymers. Following *in vivo* administration as a mouthwash, these polymers persisted upon the human buccal mucosa for at least one hour. This method is only suitable for assessing the liquid dosage forms, which are widely employed to enhance oral hygiene and to treat local disease conditions of the mouth such as oral candidacies and dental caries [91].

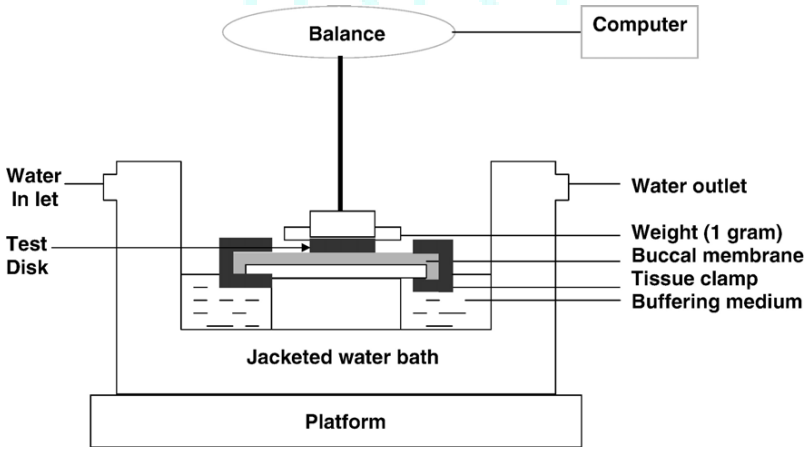

Figure 4 Schematic representations of apparatus for measuring tensile strength

Qualitative methods

These methods are useful for preliminary screening of the respective polymer for its bio or mucoadhesion, compatibility and stability. However, these methods are not useful in measuring the actual bioadhesive strength of the polymers. They are [92]

Viscometric method

Katarina Edsman has studied the dynamic rheological measurements on gels containing four different carbopol polymers and the corresponding mixtures with porcine gastric mucin and bovine submaxillary mucin. The method does not give the same ranking order when two different comparison strategies were used. The results were contrast to the results

obtained with the tensile strengthmeasurements.

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 50 MDa. Since complexes can be very large, a more sensible assay procedure is to use sedimentation velocity with change in sedimentation coefficient, *s*, as their marker for mucoadhesion. Where mucin is available in only miniscule amounts, a special procedure known as Sedimentation Fingerprint- ing 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 ratio ($s_{complex}/s_{mucin}$), the ratio of the sedimentation coef- ficient of any complex involving the mucin to that of pure mucin itself, is used as the measure for mucoadhesion.

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 a higher surface roughness.

Electrical conductance

Bremakar 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 labeled with pyrene and fluorescein 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 adhesion.

Lectin binding inhibition technique

The method involves an avidinbiotin 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 cancanavalian 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 glycoconjugates, thus reducing or inhibiting cancanavalian A binding.

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.

Factors affecting bio/mucoadhesion

Numerous studies have indicated that there is certain molecular weight at which bioadhesion is optimum. The optimum molecular weight for the maximum bioadhesion depends on the type of polymers. It dictates the degree of swelling in water, which in turn determines interpenetration of polymer molecules within the mucus. It seems that the bioadhesive force increases with the molecular weight up to 100,000 and beyond this level there is not much effect. For the best bioadhesion to occur, the con- centration of polymer must be at optimum. Flexibility of polymer chain is also important for interpenetration and entanglement. As water-soluble polymers become cross-linked, the mobility of the individual polymer chain decreases. As the cross linking density increases, the effective length of the chain, which can penetrate into the mucus layer, decreases even further and mucoadhesive strength is reduced. Besides molecular weight or chain length, spatial conformation of a molecule is also important. Despite a high molecular weight of 19,500,000 for dextrans, they have similar adhesive strength to that of polyethylene glycol with a molecular weight of 200,000. The helical conformation of dextran may shield many adhesively active groups, primarily responsible for adhesion, unlike PEG polymers, which have a linear conformation. Swelling is not only related to the polymer itself, and also to its environment. Interpenetration of chains is easier as polymer chains are disentangled and free of interactions. Swelling depends both on polymer concentration and the presence of water. When swelling is too great, a decrease in bioadhesion occurs [93].

IV. DEVELOPMENTS IN BUCCAL ADHESIVE DRUG DELIVERY

Retentive buccal mucoadhesive formulations may prove to be an alternative to the conventional oral medications as they can be readily attached to the buccal cavity retained for a longer period of time and removed at any time. Buccal adhesive drug delivery systems using matrix tablets, films, layered systems, discs, micro spheres, ointments and hydrogel systems has been studied and reported by several research groups. However, limited studies exist on novel devices that are superior to those of conventional buccal adhesive systems for the delivery of therapeutic agents through buccal mucosa. A number of formulation and processing factors can influence properties and release properties of the buccal ad- hesive system. There are numerous important considerations that include biocompatibility (both the drug/device and device/ environment interfaces), reliability, durability; environmental sta- bility, accuracy, delivery scalability and permeability are to be considered while developing such formulations. While biocom patibility is always an important consideration, other considerations vary in importance depending on the device application. Bioadhesive formulations designed for buccal application should exhibit suitable rheological and mechanical properties, including pseudo- plastic or plastic flow with thixotrophy, ease of application, good spreadability, appropriate hardness, and prolonged residence time in the oral cavity. These properties may affect the ultimate perfor- mance of the preparations and their acceptance bypatients[94].

Research on buccal adhesive drug delivery systems

Several buccal adhesive delivery devices were developed at the laboratory scale by many researchers either for local or systemic actions. They are broadly classified in to Solid buccal adhesive dosage forms, Semi-solid buccal adhesive dosage forms and Liquid buccal adhesive dosage forms [95] .

V. EVALUATION

In addition to the routine evaluation tests such as weight variation, friability, hardness, content uniformity, *in vitro* dissolution for tablets; tensile strength, film endurance, hygroscopicity etc for films and patches; viscosity, effect of aging etc for gels and ointments; buccal adhesive drug delivery devices are also to be evaluated specifically for their mucoadhesive strength and permeability.

Determination of the residence time [96]

***In vitro* residence time**

It was determined using a modified USP disintegration apparatus as shown in Figure 5. The disintegration medium composed of 800 ml isotonic phosphate buffer pH 6.75 maintained at 37 °C. A segment of rabbit intestinal mucosa, 3 cm long, was glued to the surface of a glass slab, vertically attached to the apparatus. The mucoadhesive tablet was hydrated from one surface using 15 ml IPB and then the hydrated surface was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down so that the tablet was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the tablet from the mucosal surface was recorded.

***In vivo* residence time test**

The experiment was conducted on four human healthy volunteers of 25–50 years old. Plain bioadhesive tablets with optimized properties were selected for the *in vivo*. The bioadhesive tablet was placed on the buccal mucosa between the cheek and gingiva in the region of the upper canine and gently pressed onto the mucosa for about 30 s. The tablet and the inner upper lip were carefully moistened with saliva to prevent the sticking of the tablet to the lip. The volunteers were asked to monitor the easewith which the system was retained on themucosa and note any tendency for detachment. The time necessary for complete erosion of the tablet was simultaneously monitored by carefully observing for residual polymer on the mucosa. In addition, any complaints such as discomfort, bad taste, dry mouth or increase of salivary flux, difficulty in speaking, irritation or mucosal lesions were carefully recorded. Repeated application of the bioadhesive tablets was allowed after a two days period for the same volunteer.

Permeation studies

During the preformulation studies, buccal absorption/ permeation studies must be conducted to determine the feasibility of this route of administration for the candidate drug and to determine the type of enhancer and its concentration required to control the rate of permeation of drugs. These studies involvemethods that would examine in *vitro, ex vivo* and/or *in vivo* buccal permeation profile and absorption kinetics of the drug.

***In vitro* methods** [97]

Deasy used an apparatus consisting of a water jacket and an internal compartment containing 50 ml of simulated saliva as dissolution medium to study the release of cetylpyridinium chloride tablet by placing in the metal die sealed at the lower end by paraffin wax to ensure the drug release from one end alone. The medium was stirred with a rotating stirrer at 250 rpm. Ishida conducted dissolution studies with similar apparatus with slight modification of providing a water jacket for the maintenance of temperature for dosage forms of lidocaine. Nagai used Toyamp-Sangyo TR-553 dissolution tester to measure the dissolution rate of disk like dosage forms by keeping in a rotating basket at 100 rpm in 900 ml of purified water. The same apparatus was used for the evaluation of oral mucosal dosage forms of insulin. Hughes and Gehris described a novel dissolution testing system that is capable of characterizing buccal dissolution.

It comprises of a single, stirred, continuous flow-through filtration cell that includes a dip tube designed to remove finely divided solid particles. Filtered solution is removed continuously and used to analyze for dissolved drug.

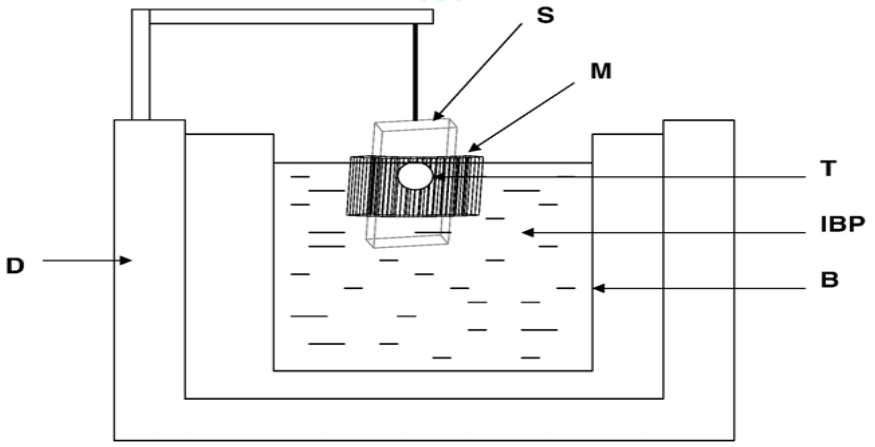

Figure 5 Schematic diagram of the apparatus used for the determination of residence time. S: glass slab; D: disintegration apparatus; B: glass beaker; M: mucosal membrane; T: mucoadhesive tablet; IBP: Isotonic phosphate buffer.

***Ex vivo* methods** [98]

Most of the *ex vivo* studies examining drug transport across buccal mucosa uses buccal tissues from animal models. Immediately after sacrificing the animals the buccal mucosal tissue is surgically removed from the oral cavity. The membranes are stored in Krebs buffer at 4 °C until mounted in the diffusion cells for the *ex vivo* permeation experiments. Preservation of the dissected tissue is an important issue that will affect the studies.

There is no standard means by which the viability or the integrity of the dissected tissue can be assessed. The most meaningful method to assess tissue viability is the actual permeation experiment itself, if the drug permeability does not change during the time course of the study under the specific experimental conditions of pH and temperature, then the tissue is considered viable.

Dowty] studied tissue viability by using ATP levels in rabbit buccal mucosa. He reported a 50% drop in the tissue ATP concentration during the initial 6 h of the experiment without a corresponding drop in tissue permeability. Despite certain gradual changes, the buccal tissue seems to remain viable for a rather long period of time. Hence, a decrease in ATP levels does not assure a drop in permeability characteristics of the tissue.

Buccal cell cultures have also been suggested as useful in *vitro* models for buccal drug permeation and metabolism. However, to utilize these culture cells for buccal drug transport, the number of differentiated cell layers and the lipid composition of the barrier layers must be well characterized and controlled.

***In vivo* methods** [99]

Selection of animal species

Apart from the specific methodology used to study buccal drug permeation characteristics, special attention is warranted to the selection of experimental animal species for such experiments. Many researchers have used small animals including rats and hamsters for permeability studies. But unlike humans, most laboratory animals have totally keratinized oral lining, hence not suitable. The rat has a buccal mucosa with a very thick, keratinized surface layer. The rabbit is the only laboratory rodent that has non-keratinized mucosal lining similar to human tissue. But, the sudden transition to keratinized tissue at the mucosal margins makes it hard to isolate the desired non-keratinized region.

Buccal absorption test.

Beckett and Triggs developed a method to measure the kinetics of drug absorption. It is carried out by swirling of a 25 ml sample of the test solution for 15 min by human volunteers followed by the expulsion of the solution. The amount of drug remaining in the expelled volume is then determined to assess the amount of drug absorbed. The drawbacks of this method are inability to localize the drug solution within a specific site of the oral cavity, accidental swallowing of a portion of the sample solution and the salivary dilution of the drug.

Modified buccal absorption test

Gonzalez-Younes et al. developed this method by correcting for salivary dilution and accidental swallowing, but these modifications also suffer from the inability of site localization.

Perfusion system

A circulating perfusion chamber attached to the upper lip of anesthetized dogs by cyanoacrylate cement and the drug solution is circulated through the device for a predetermined period of time. Sample fractions are collected from the perfusion chamber and blood samples are drawn at regular intervals.

Buccal perfusion cell apparatus [100]

Rathbone developed an apparatus that provides continuous monitoring of drug loss as a function of time offers larger area for drug transfer and has no leakage problem. He used several methods to study the rate and extent of drug loss from human oral cavity. These include the buccal absorption test, disk methods and perfusion cells. These methods have provided information on the mechanism by which drugs are transported across oral cavity membranes and suggest that passive diffusion or carrier mediated transport systems may be involved. *In vivo* buccal permeation of FITC labeled dextran 4400 and the peptide drug buserelin was investigated in pigs. The delivery device consisted of an application chamber with a solution of FD4 or buserelin, and was attached to the buccal mucosa for four hours using an adhesive patch. The randomized crossover study including intravenous administration and buccal delivery without and with 10 mM sodium glycodeoxycholate as an absorption enhancer was performed in pigs.

VI. CONCLUSION

For research it is the need to drug delivery systems extends beyond ways to administer new pharmaceutical therapies. The safety and efficacy of current treatments may be improved if their delivery rates, biodegradation and site specific targeting can be predicted, monitored and controlled. Buccal adhesive systems offer innumerable advantages in terms of accessibility, administration and withdrawal, retentivity, low enzymatic activity, economy and high patient compliance. Since the introduction of Orabase in 1947, when gum tragacanth was mixed with dental adhesive powder to apply penicillin to the oral mucosa; the market share of bioadhesive drug delivery systems is increasing. In addition, buccal adhesive dosage forms have been used to target local disorders at the mucosal surface (e.g., mouth ulcers) to reduce the overall dosage required and minimize side effects that may be caused by systemic administration of drugs. Researchers are now looking beyond traditional polymer networks to find other innovative drug transport systems.

At the current global scenario, scientists are finding ways to develop buccal adhesive systems through various approaches to improve the bioavailability of orally poor soluble drugs by manipulating the formulation strategies like inclusion of pH modifiers, enzyme inhibitors, permeation enhances etc. Novel buccal adhesive delivery system, where the drug delivery is directed towards buccal mucosa by protecting the local environment is also gaining interest. Currently solid dosage forms, liquids and gels applied to oral cavity are commercially successful. The future direction of buccal adhesive drug delivery lies in vaccine formulations and delivery of small proteins/peptides. Microparticulate bioadhesive systems are particularly interesting as they offer protection to therapeutic entities as well as the enhanced absorption that result from increased contact time provided by the bioadhesive component.

VII. REFERENCES

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