CHAPTER II
LITERATURES REVIEW

Oral cavity as site for bioadhesive drug delivery

Attention in the area of soft tissue based mucosal delivery, and several formulations are now commercially available or under development. Such systems dramatically increase dosage form residence time, as well as improve intimacy of contact with the tissue, thereby localizing the drug in a specific region. Bioadhesion, therefore, has the potential to maintain the dosage form for a clearly defined time on the oral mucosa (Rajesh and Joseph, 1994).

Bioadhesion is an interfacial phenomenon in which two materials, at least one of which is biological, are held together by means of interfacial forces. The attachment is typically between an artificial material and a biological substrate, such as adhesion between a polymer and/or copolymer and a biological membrane. In the case of a polymer attached to the mucin layer of mucosal tissue, the term mucoadhesion is employed. The use of bioadhesive drug delivery systems for local and systemic delivery via the oral cavity, will be discussed in this review.

The delivery of therapeutic agents, for both local and systemic delivery, via the oral mucosa offers a number of advantages over conventional routes. The oral cavity is convenient and easily accessible. This route is expected to have a higher level of patient compliance than parenteral or rectal routes. Enzyme and acid mediated flux degradation and "first pass metabolism", the two major barriers associated with conventional oral administration, can be avoided via this route. Circulation in the oral cavity mucosa is drained by the internal jugular vein, thus absorbed drugs will enter the systemic circulation directly and will bypass first pass liver metabolism.

Localization of drugs and other formulation adjuvants is possible. Thus, protease inhibitors or penetration enhancers can be incorporated to locally modify the tissue and enhance permeability.

Overview of oral mucosa

The oral mucosa is a complex series of tissues demonstrating a range of permeabilities. The differences in barrier properties reflect the structure of the oral lining in different regions of the mouth. It is useful to briefly review the oral mucosa structure relevant to drug delivery.

1. Structure of the oral mucosa

The oral cavity is lined with stratified squamous epithelium, below which lies the basement membrane, supported by a connective tissue lamina propria as shown in figure 1. Classification of epithelium into recognizable cell layers is usually difficult because well defined strata, as seen in the epidermis or keratinized oral epithelium, are absent. There is a well defined layer of basal cuboidal cells succeeded by several rows of slightly flattende cells representing the prickle cell layer. This layer is also referred to as the spinous cell layer. The prickle cell layer is further divided into the upper (superficial) and lower (basal) prickle cells. The remaining one-third of the epithelium consists of flattened nucleated cells called superficial cells. Beneath the epithelium is the underlying connective tissue, referred to as lamina propria. The lamina propria consists of collagen fibers, a supporting layer of connective tissue, blood vessels, and smooth muscle. Drugs administered via the oral mucosa gain access to the systemic circulation through a network of arteries and capillaries. As shown in figure 2, the gingiva and the hard palate are lined with a masticatory mucosa, where the epithelium has a cornified surface containing keratin. The labial and buccal mucosa, floor of the mouth, soft palate, and underside of the tongue are lined with non-keratinized stratified squamous epithelium. These regions represent the major absorption site in the oral cavity.

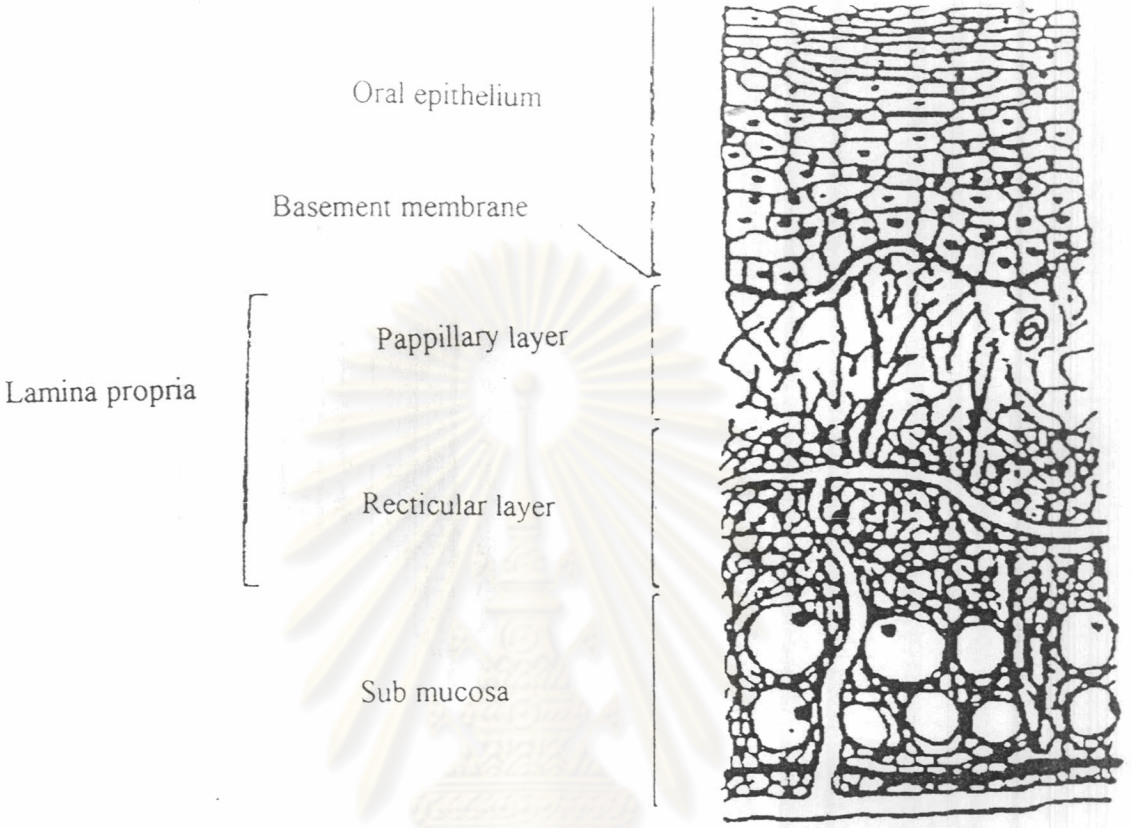

Figure 1 Schematic diagram showing the main tissue components of the oral mucosa.

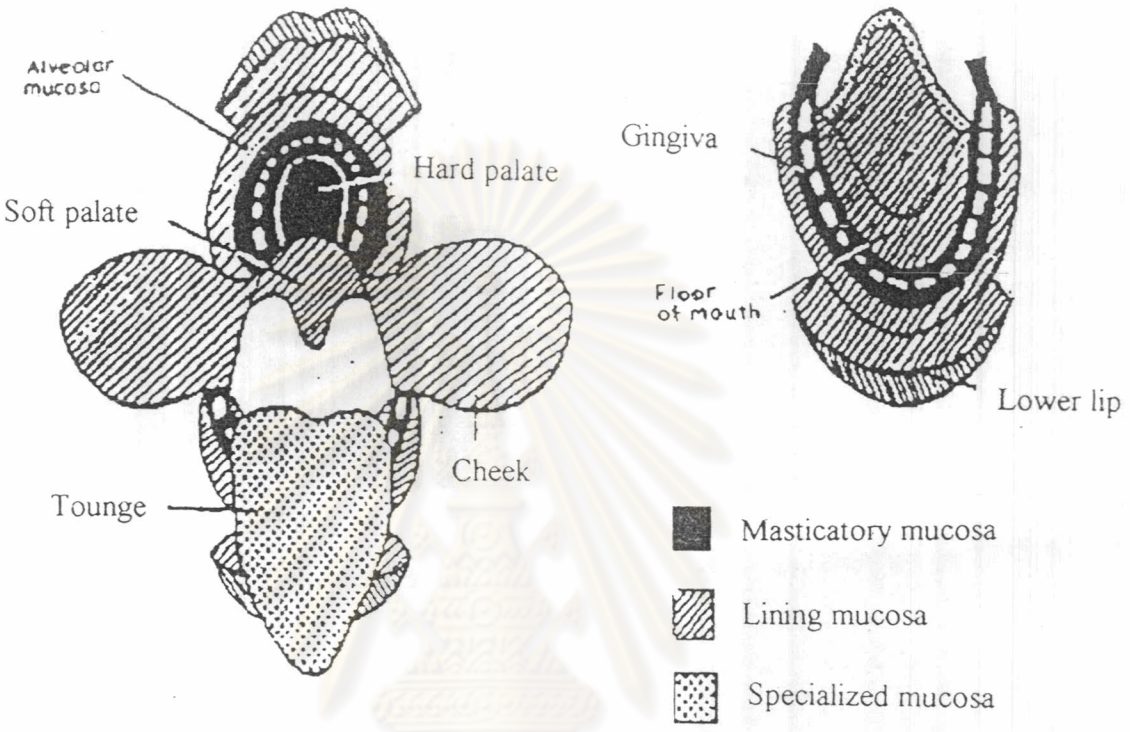

Figure 2 The oral cavity showing the regions occupied by masticatory, lining, and specialized mucosa.

The thickness of the oral epithelium varies depending on the location and species. In humans, dogs, and rabbits, the buccal mucosa measures 500-800 μm in thickness, whereas the floor of the mouth, ventral tongue, and gingiva region measure 100-200 μm. The surface of the mucus membrane is continuously washed by a stream of about 0.5 to 2 liters of saliva daily.

The composition of the epithelia varies depending on location. For a detailed discussion of biochemistry of the oral mucosa, the reader is referred to the review by Gerson and Harris (Rajesh, et al., 1994). Cells of both keratinized and non-keratinzed epithelia contain large amounts of proteins, in the form of tonofilaments. Cells of non-keratinized epithelia contain low molecular weight proteins whereas those of keratinized epithelia contain higher molecular weight keratin. The keratinized epithelia contain neutral lipids like ceramides and acyl ceramides which have been associated with the barrier function (Yardley and Summerly, 1981 and Wertz, et al., 1986). These epithelia are relatively impermeable to water. In contrast, non-keratinized epithelia, such as the floor of the mouth and the buccal epithelia, do not contain acylceramides and possess only small amounts of ceramide. They contain few neutral but polar lipids, particularly cholesterol sulfate and glucosyl ceramides (Squier, et al., 1986 and Curatolo, 1987). These epithelia have been found to be considerably more permeable to water than keratinized epithelia (Squier and Hall, 1985).

2. Permeability and permeability barriers of the oral mucosa

One of the fundamental properties of the oral mucosa is its barrier function. It excludes potentially dangerous endogenous or exogenous substances present in the oral cavity. Like the skin, the oral mucosa consists of stratified squamous epithelium. However, unlike the skin, which has a dry surface coated with sebaceous lipid, the oral mucosa is always moist because of saliva that is constantly secreted and it does not show the presence of keratin (buccal and sublingual). These dissimilarities with skin make the oral mucosa more permeable than skin. The buccal permeability value for water (Lesch, et al., 1980) suggests the permeability coefficient (K_p) values for the oral mucosa to be greater than that for skin. This improved permeability of the oral

mucosa, as compared to skin, holds for a wide variety of drugs. Both the gut and the oral mucosa are kept moist all the time. However, the gastrointestinal tract is lined with columnar epithelia, highly specialized for its absorptive function. Hence, one might expect the oral mucosa to be less permeable than the gut and to have permeability characteristics between that of the gut and skin and closer to the gut than skin.

2.1 Experimental systems

The techniques to measure permeability have been reviewed by Harris and Robinson (Harris and Robinson, 1992) and Rathbone and Hadgraft (Rathbone and Hadgraft, 1991). A simple non-invasive method to determine the permeability of drugs across oral mucosa was introduced by Beckett and Triggs (Beckett and Triggs, 1967). In this method, a solution of known concentration of drug is swirled around the mouth by movement of the cheeks and tongue for a defined length of time. The solution is expelled and subsequently analyzed for its drug content. The amount of drug absorbed is then calculated as the difference between the amount contained in the original buffered drug solution and the amount recovered. Several pre- and post-test modifications of this method exist in the literature, and have been reviewed by Rathbone and Hadgraft (Rathbone and Hadgraft, 1991). The major disadvantage of this method is that it does not provide information on relative permeabilities of different areas of the oral cavity and there is no control over the area of absorption, which may result in considerable inter-subject variations.

Another method was devised by Kaaber (Kaaber, 1974). In this method, an airtight sampling chamber comprised of a standardized disc of dry, ash-free filter paper is overlaid with a disc of porous membrane material. Even though this technique can define the oral cavity area, it suffers from inherent disadvantages such as adherence of the disc to the membrane, leakage of drug from the disc, and interference from salivary secretions.

Some of the limitations described in the above method can be overcome by using in-situ perfusion cells or a similar device that is either clamped or attached to oral mucosa (Barsuhn, et al., 1988, Veillard, et al., 1987, Zhang et al., 1989 and

Yamahara, et al., 1990). This method measures absorption by measuring disappearance from perfusate, appearance in bloodstream, or pharmacologic response and the technique permits isolation of the area of interest within the oral cavity. The main drawback with the perfusion cell is leakage and large inter-subject variation. Recently, Rathbone (Rathbone, 1991) reported an improved buccal perfusion cell design eliminating the leakage problem, maintaining low perfusion circuit pressure, and reducing intra- and inter-subject variations.

The most recent technique to measure cell permeability involves the use of cell or tissue cultures (Tavakoli-Saberi and Audus, 1989). The cell differentiation observed in cultured cells is not identical to that observed in intact tissues. However, with the development of tissue culture techniques, it appears that this technique may be, by far, the most useful technique to study transport phenomena at the cellular and subcellular levels.

2.2 Permeability barrier

It is currently believed that the permeability barrier in the oral mucosa is a result of intercellular material derived from the so-called "membrane coating granules (MCGs)". This barrier exists in the outermost 200 μm of the superficial layer. Permeability studies have been performed using two tracers, namely, lanthanum nitrate and horseradish peroxidase. The two tracers differ in size and chemical properties. Horseradish peroxidase is a macromolecule (M_r 40,000), 5-6 nm in size, while lanthanum exists as a colloid, 2 nm in size. Even though the two tracers have different sizes, both are hydrophilic and are therefor, expected to be confined to aqueous pathways through the mucosa. Topically applied tracers did not penetrate further than the top 1-3 cell layers. However, when these same probes were introduced subepithelially, they extended through the intercellular spaces into the prickle cell layers. In both keratinized and non-keratinized epithelium, the limit of penetration coincided with the level where the MCGs could be seen adjacent to the superficial plasma membranes of the epithelial cells. Since the pattern of penetration is similar in both keratinized and non-keratinized epithelia, it is unlikely the keratinization, is a major barrier for penetration. Since the limit of penetration

coincided with the levels where the MCGs are seen, it appears that MCGs are involved in formation of the major barrier for penetration. Microscopically visible tracers like horseradish peroxidase and lanthanum can provide useful information on the site and extent of the barrier in the epithelium. Autoradiography studies of small-molecular-weight peptides confirm that only the outer one-third of the epithelial tissue is rate limiting and no barrier properties are found beneath this layer.

MCGs are spherical or oval organelles, about 100-300 nm in diameter, found in many stratified epithelia (Maltoltsy, 1976). The term membrane coating granule was based on the view that disappearance of the granules from the cytoplasm was followed by, and functionally related to, thickening of the cell plasma membrane. The process of membrane thickening is better understood now and is apparently due to accretion of unidentified material onto the cytoplasmic surface of the membrane. MCGs first appears in cells of the stratified spinosum of the keratinized epithelia, and at about the same distance from the basal cells in non-keratinized epithelia. The majority of these granules appear along the upper distal or superficial border of each cell. As differentiation proceeds, they are discharged into the intercellular spaces by exocytosis, with the membrane of the MCG incorporated into the cell membrane. Among epithelial cells, MCGs are found in almost all stratified squamous epithelia. Their presence does not appear to depend on the existence or degree of keratinization. The number of granules in the cells appear to depend on the body region, and differ between various sites within the keratinized or non-keratinized epithelia. In the MCGs of keratinized epithelia, there is a complex internal structure of parallel lamellation which consists of alternating electron-dense and electron-translucent bands. However, MCGs of non-keratinized epithelia usually do not possess a lamella internal structure (Silverman and Kearns, 1970). The granules are enclosed in a trilaminar membrane, but the contents are finely granular and aggregate centrally, so as to leave a clear zone beneath the limiting membrane. The work on these granules has been mostly structural. MCGs of keratinized epithelia have been shown to contain polar lipids (glycolipids and phospholipids), glycoproteins, and a number of hydrolytic enzymes. Characterization of the enzyme content of the fraction revealed it to be rich in acid phosphatase, carboxy peptidase, cathepsin-B, acid lipase, sphingomyelinase, and phospholipase. However, the enzyme content was strikingly depleted in all sulfatase, β-glucuronidase, and the non-lysosomal protease (Grayson, et al., 1985).

It has been shown that for some compounds the barrier to penetration is not the upper one-third of the epithelium. Alfano and his co-workers (Alfano, et al., 1975) studied the penetration of endotoxins through non-keratinized oral mucosa. The results of their studies indicated that the basement membrane is a rate limiting barrier to permeation. The structure of the lamina propria is not sufficiently dense to present a barrier to permeation of relatively large molecules. Hence with few exceptions most studies reveal that the outer one-third of the epithelial tissue is rate limiting to permeation.

2.3 Mechanism of drug transport

Substances can be transported across various epithelial membranes by means of simple diffusion, carrier mediated diffusion, active transport or other specialized mechanisms, such as endocytosis. While cells of the oral epithelium and epidermis are capable of taking up materials by endocytosis, particularly in the basal and prickle layers, it does not seem likely to be a transport mechanism across an entire stratified epithelium. There is considerable evidence that most substances passing across the oral mucosa move by simple Fickian diffusion. The early works of Beckett and his co-workers indicated that loss of drugs from the oral cavity occurred by the process of passive diffusion of the non-ionized form in accordance with the pH-partition hypothesis. Some amino acids, like glutamic acid and lysine, are reported to be transported via a carrier-mediated process. Also, certain vitamins, like L-ascorbic acid (Sadoogh-Abasian and Evered, 1979), nicotinic acid (Evered, et al., 1980), and thiamine (Evered and Mallett, 1983), are transported via carrier-mediated transport. While both glutathione and homocitrulline are transported by a carrier-mediated process, the transport of the former is sodium independent and that of the latter is sodium dependent. A few monosaccharides (Evered, et al., 1980) have been proven to be transported by a carrier-mediated process.

Two potential routes across the oral mucosa can be classified as non-polar and polar. The non-polar route involves lipid elements of the mucosa by partitioning of the drug into the lipid bilayer of the plasma membrane or into the lipid of the intercellular matrix. The polar route involves the passage of hydrophilic material

through aqueous pores in the plasma membrane of individual epithelial cells, or ionic channels in the intercellular spaces of the epithelium. The rate at which a given substance will pass across the oral mucosa is determined by its partitioning between the lipid and water (Schanker, 1964). Substances with high lipid solubility will be transported across the lipid rich plasma membranes of the epithelial cells, while water soluble substances will pass through the intercellular spaces. An alternative classification involves passage through intercellular spaces between the cells, i.e., the paracellular route and transport into and across the cells i.e., the transcellular route. The transcellular route involves partitioning, cellular channel diffusion, and carrier mediated transport. However, the paracellular route represents diffusive convective transport occurring through the intercellular space.

Some of the electrical properties that are used to classify epithelia as leaky or tight include the measurement of resistance or potential difference (Fromter and Diamond, 1972). Accordingly, tissues like the rabbit gall bladder, rat duodenum and jejunum (Okada, et al., 1977), and rabbit ileum with low resistance and potential difference are classified as leaky, and hence, more permeable. The gastric mucosa of the fundus of nectrus (Spenney, et al., 1974), frog skin, toad bladder epithelium, and the rabbit buccal (Gandhi and Robinson, 1991) with high resistance and potential difference are classified as tight mucosa. In terms of better permeability, the nasal, rectal, and vaginal mucosa appear to be preferred over the oral area (buccal and sublingual). However, because of excellent accessibility of the oral mucosa, appropriate dosage forms can be fabricated and drug action can be terminated at any time by simply removing the dosage form. For this route, patients are expected to have high compliance and the application is essentially painless. Also, according to the natural function of the oral mucosa, it is routinely exposed to a multitude of foreign substances and, hence, must be rather robust and less prone to irreversible damage by drug, dosage form, adjuvants like penetration enhancers, enzyme inhibitors, and/or solubilizers. Thus, in spite of undoubtedly higher natural permeability of most other routes, the buccal area appears attractive for delivery of certain drugs, particularly if bioadhesives are part of the delivery system.

Factor affecting bioadhesive

Formation of an adhesive bond between a polymer and biological membrane, or its coating, can be visualized as a two step process. The first step involves initial contact between the two surfaces. The second step involves formation of secondary bonds due to non covalent interactions. The surface of the biological membrane, and the surface of the adhesive, form an interfacial layer between the two surfaces and this interface causes bond formation. In most cases, the adhesive interaction would initially be between the bioadhesive polymer and the mucus layer, and would not directly involve the epithelial surface. Molecular events that take place in the interfacial layer depend on various characteristics of both the polymer and the membrane. In order to gain a thorough understanding of interfacial events, a brief discussion of the characteristics of the two surfaces will be presented.

1. Biological membrane

The oral cavity is covered with a gel-like structure known as mucus. Hence, during the process of attachment, all bioadhesive materials must interact with the mucus layer. Mucus serves as a link between the adhesive and the membrane. The composition of mucus varies widely, depending on animal species, anatomical location, and whether the tissue is in a normal or pathological state. There is considerable variation in thickness and composition of the mucus layer within the oral mucosa. Mucus is synthesized either by goblet cells lining the epithelia or by special exocrine glands with mucus cells (Schachter and Williams, 1982). Mucus secreting glands contain a precursor of mucus called mucinogen, which is released on the surface of the epithelium.

Mucus is a glycoprotein, chemically consisting of a large peptide backbone with pendant oligosaccharide side chains many of which terminate in either sialic acid or sulfonic acid, or L-fucose (Beyer, et al., 1979). The oligosaccharide chains are covalently linked to the hydroxyl amino acids, serine and threonine, along the polypeptide backbone (Ginsburg and Neufeld, 1969) as shown in figure 3. About 25% of the polypeptide backbone is without sugars, the so called "naked" protein region, which is especially prone to enzymatic cleavage (Silberberg and Meyer, 1982,

Kandukuri, 1977, and Scawen and Allen, 1977). This region, being rich in charged amino acids, chiefly aspartic acid is involved in cross-linking via disulfide bonds between mucin molecules (Scawen and Allen, 1977, and Mantle and Allen, 1981). The remaining 75% of the backbone is heavily glycosylated. A highly extended and flexible molecular conformation is suggested for mucus glycoproteins (Morris and Rees, 1978) to permit maximum ability to sorb water. The terminal sialic acid groups have a pK_a value of 2.6 (Kornfeld, 1976). Therefore, the mucin molecule should be viewed as a polyelectrolyte under neutral or slightly acid conditions. At physiological pH, the mucus network may carry a significant negative charge because of the presence of sialic acid and sulfate residues, and this high charge density due to negative charges contributes significantly to bioadhesion. The entangled nature of mucus is due to disulfide linkages, physical entanglement, and secondary bonds, e.g., electrostatic, hydrogen bonding, and hydrophobic interactions (Silberberg and Meyer, 1982). Hence, mucus can be viewed as a macromolecular association linked together via cross-linking, which gives rise to an aggregated structure. The glycoprotein fraction of the mucus imparts a viscous or gel-like characteristic to mucus due to its water retention capacity, i.e., it holds about 40-times its weight of water. Under physiological conditions, the glycoprotein is capable of a wide range of rheological behavior, including gel formation. Mucus has strong cohesive properties and firmly binds to the epithelial cell surface as a continuous gel layer, and the gel obviously behaves as a non-Newtonian fluid (Snary, et al., 1971). The concentration of mucus in solution may be the most important parameter in determining its rheological properties. Involvement of the epithelial cell layer, the structure and density of the cell surface oligosaccharide side chains and their interaction with lipids and proteins must be considered in developing an accurate mechanism of bioadhesion. Adhesive properties of cells may also be due to their "fuzzy coat" glycocalyx, which consists of polysaccharide containing structures external to the cell surface. In addition mucus contains water, electrolytes, sloughed epithelial cells, enzymes, bacteria, bacterial byproducts, and other debris (Schachter and William, 1982).

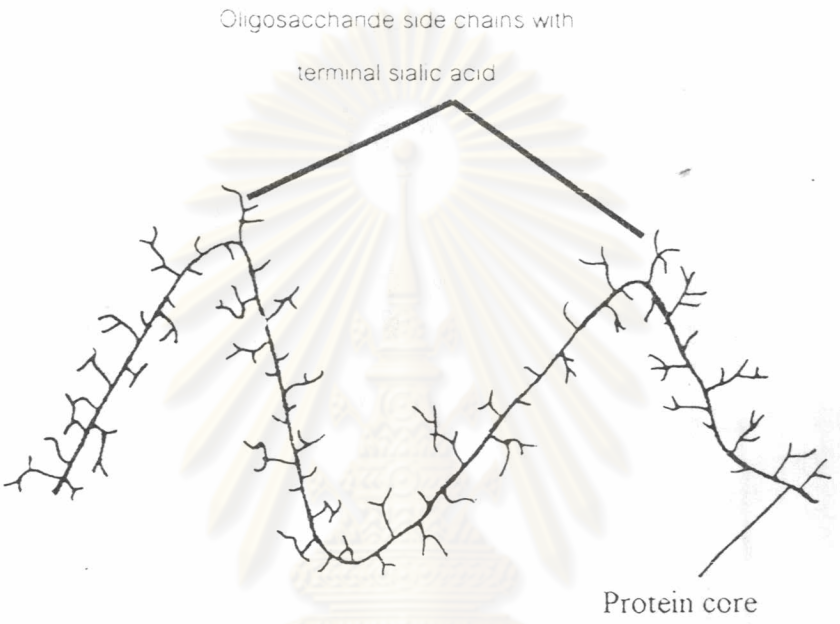

Figure 3 Schematic structure of mucin

2. Polymer-related factors

Park and Robinson (Park and Robinson, 1986) have listed the polymers that are currently used in bioadhesive systems. It appears that a variety of polymers can be used, including many water-soluble and insoluble hydrocolloid polymers, both ionic and non-ionic, as well as insoluble hydrogels. Drug release from soluble polymers typically occurs by bulk erosion. However, drug release from insoluble hydrogels follows either Fickian or non-Fickian diffusion kinetics. The bioadhesive properties of a polymer is affected by the following factors.

2.1 Molecular weight

As mentioned by Gurny, Meyer, and Pappas (1984), it seems that the bioadhesive force increases with the molecular weight of bioadhesive polymer, up to 1,000,000 and beyond this level there is not much effect. It clear that, to allow chain interpenetration, the polymer molecule must have an adequate length. It is also necessary to consider the size and configulation of the polymer molecule. Besides molecular weight, spatial conformation of the 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 200,000. The helical conformation of dextran may shield many adhesively active groups unlike polyethylene glycol polymers which have a linear conformation (Gandhi and Robinson, 1988).

2.2 Concentration of the polymer

In order to achieve optimal bioadhesion, there exists a critical concentration of polymer (Gurny, et al., 1984). When the concentration of polymer increases, the adhesive strength decreases significantly. In a concentrated solution of a polymer, the coiled molecules become solvent-poor. As a result, the macromolecules approach the dimension of an unperturbed state, and the available chain length for penetration decreases.

2.3 Swelling

This characteristic is related to the polymer itself, and also to it environment. Swelling depends both on polymer concentration and on water presence. It must be remembered that, when swelling is too great, a decreasing in bioadhesion occurs due to formation of slippery.

3. Environment-related factors

3.1 pH

pH was also found to have a significant effect on mucoadhesion as observed in studies of polyacrylic acid polymers cross-linked with carboxyl groups (Park and Robinson, 1984). pH influences the charge on the surface of both mucus and the polymer. Mucus will have a different charge density on the surface depending on pH because of the difference in dissociation of functional groups on the carbohydrate moiety and amino acid moiety of the polypeptide backbone. Robinson and his group observed that the pH of the medium is critical for hydration of the lightly cross-linked polyacrylic acid copolymers. The apparent pK_a for the polymer is approximately 4.7 (Ch'ng, et al., 1985). Maximum adhesion was observed at pH 5 and 6 and a minimum at pH 7. This behavior was attributed to difference in charge density at different pHs. Hence, the charge density of both mucin and the polymer are influenced by pH, which in turn affects bioadhesion.

3.2 Hydration

The presence of fixed charges originating within the macromolecular network establishes a swelling force or net osmotic pressure. This drives the solvent into the polymer gel from the more dilute external bulk solution. The presence of counter-ions reduces the fixed charges on the polymer. Hence, the presence of ionic species in a polymer solution can significantly influence bioadhesive strength. The swelling state of the polymer contributes to its bioadhesive behavior. The general belief that increased swelling increases bioadhesive strength is not true, as is evidenced by the decrease in adhesion above the pK_a for the lightly cross-linked

polyacrylic acid. Maximum swelling occurs above the pK_a . For example, in the case of Orabase®, the wet adhesive strength increases with an increased degree of swelling. However, excess water results in an abrupt drop in adhesive strength and thus, adhesive strength is optimum at a certain degree of hydration. Sufficient water is necessary to hydrate the mucoadhesive to expose the adhesive site for secondary bond formation, expand the gel to create pores of sufficient size, and mobilize all the flexible polymer chains for interpenetration. When the degree of hydration is high, adhesiveness is lost, probably due to formation of a slippery, nonadhesive mucilage in an environment of a large amount of water at or near the surface. Such a phenomenon must not occur too early too detach the dosage form. However, its appearance allows easy detachment of the bioadhesive system after release of the active ingredient has ceased.

Theories of bioadhesion (Gupta, et al., 1992)

The surface characteristic and composition of the mucoadhesive material, as well as, the substrate and the associated applied force to bring the substrate in contact are important parameters in assessing mucoadhesion. Bonding occurs chiefly through both physical and weak chemical bonds. Physical or mechanical bonds result from entanglement of the adhesive material and the extended mucus chains. In this regard, mutual diffusion of the polymer and mucin chains will result in maximum attachment. Chemical bonding may be of primary or secondary type. Primary bonds are due to covalent bonding and secondary bonds may be due to electrostatic, hydrophobic, or hydrogen bonds. Electrostatic interactions and hydrogen bonding appear to be important as a result of the large number of charged species e.g.,hydroxyl (-OH), carboxyl (-COOH), sulfuric acid ($-SO_3H$), and amino ($-NH_2$) groups. Hydrophobic bonding occurs when non-polar groups associate with each other in aqueous solution due to a tendency of water molecules to exclude non-polar molecules. The van der Waals attraction between hydrophobic groups have binding energies between 1-10 kcal/mol, whereas hydrogen bonds between hydrophilic groups have an energy of about 6 kcal/mol. Hydrophobic bonding is generally considered to be the most important in bioadhesion.

Several theories have been developed to describe the process involved in the formation of bioadhesive bonds. These theories have been used as guidelines in engineering possible bioadhesive drug delivery systems.

1. Wetting theory

The ability of bioadhesive polymers or mucus to spread and develop intimate contact with their corresponding substrate is one important factor for bond formation. The wetting theory, which has been used predominately in regards to liquid adhesives, use interfacial tensions to predict spread and, in turn, adhesion (Peppas and Buri, 1985, Mikos and Peppas, 1989). The structure similarities of the mucus glycoprotein and mucoadhesive, would suggest a small interfacial tension exists up on wetting which promoted interaction and the development of mucoadhesive bond (Kallaway and Warren, 1996).

Li, Bhatt, and Johnson (1998) have assessed the bioadhesive properties of several different mucoadhesive buccal patches. The results of contact-angle measurements indicated that the contact-angle decreased with an increase in amount of carbopol in the formulation. Additionally, the calculated values, using a modification of Dupre's equation, of both work of adhesion between the water and the patch (W_1) and between the patch and freshly-buccal excised rabbit buccal mucosa (W_2) increased with increase in the amount of carbopol in formulations. A correlation was found between the measured contact angle and the calculated values for W_2. The direct measurement of the force required to separate a buccal patch from excised rabbit buccal mucosa with the Instron demonstrated that the adhesive strength increased with increase in amount of carbopol. This study has shown that the measurement of contact angles alone may provide a useful technique for estimating the work of adhesion, and may serve as a convenient and rapid screening procedure to identify potential mucoadhesive buccal-patch formulations.

2. Diffusion theory

The diffusion theory suggests that interpenetration and entanglememt of bioadhesive polymer chains and mucus polymer chains produce semipermanent

adhesive bonds, and bond strength is believed to increase with the depth of penetration of polymer chains. Penetration of bioadhesive polymer chains into the network, and vise versa, is dependent on concentration gradients and diffusion coefficients. Obviously, any cross-linking of either component will tend to hinder interpenetration, but small chains and chain ends may still become entangled. It has not been determimed exactly how much interpenetration is required to produce an effective bioadhesive bond, but it is believed to be in the range of 0.2-0.5μm. And the more structurally similar a bioadhesive is to mucus, the greater the mucoadhesive bond will be.

3. Electronic theory

The electronic theory of bioadhesion was suggested by Derjaguin and Smilga. According to this theory, electron transfer occurs on contact of an adhesive polymer with a mucus glycoprotein network because of differences in their electronic structure. This results in formation of an electrical double layer at the interface. Adhesion occurs due to attractive forces across the double layer. Such a system behaves analogous to a capacitor, which is charged when two surfaces come in contact, and discharged when they are separated.

4. Fracture theory

The most useful theory for studying bioadhesion through tensile experiments has been the fracture theory, which analyzes the forces required to separate two surfaces after adhesion. Furthermore, to determine fracture properties of an adhesive union from separation experiments, failure of the adhesive must be assumed to occur at the bioadhesive interface. However, it has been demonstrated that fracture rarely, if ever, occurs at the interfacial but instead close to it (figure 4) (Ponchel, et al., 1987).

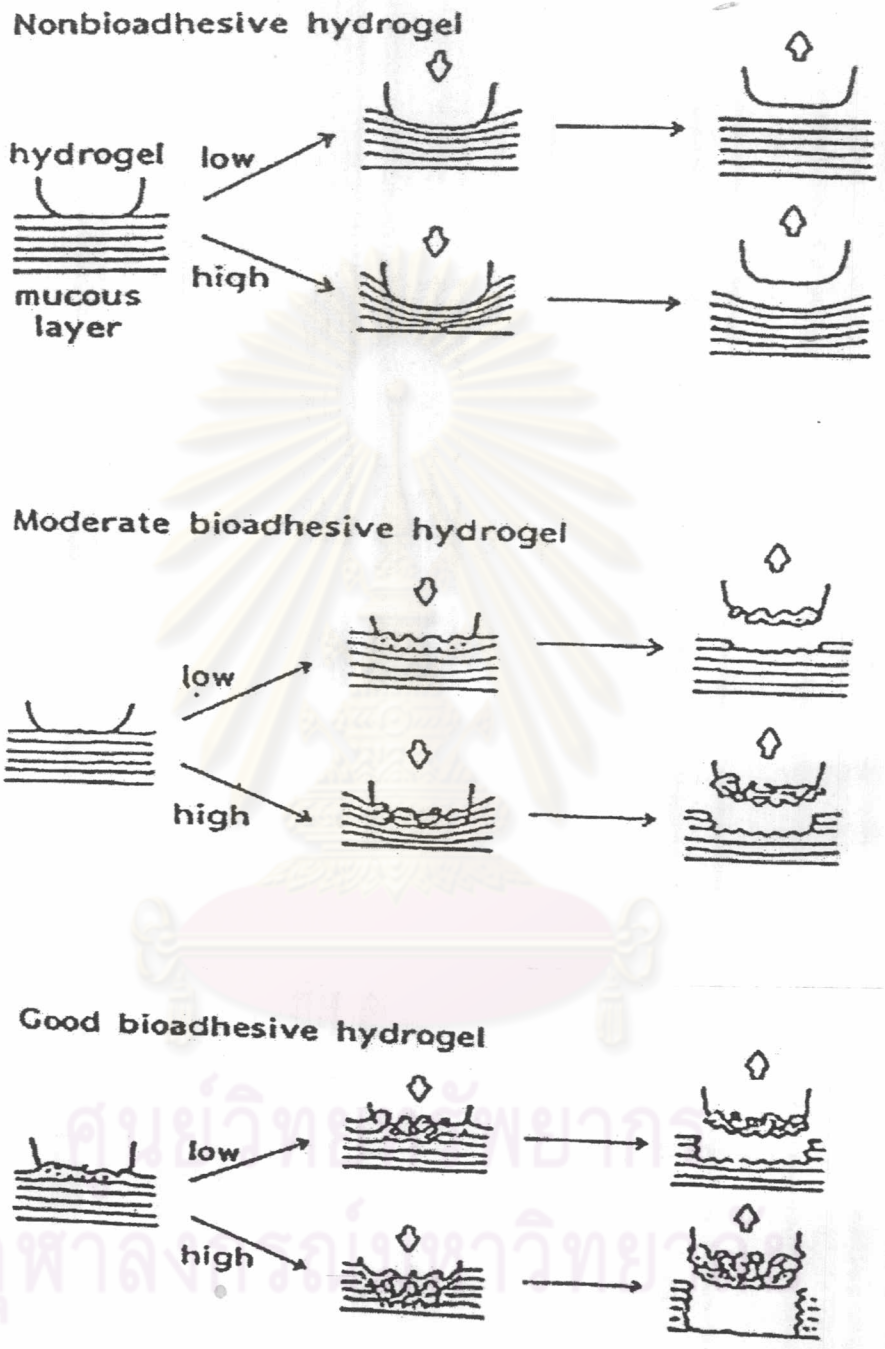

Figure 4 The interaction between mucus layers and hydrogels (Gupta, et al., 1992)

5. Adsorption theory

Adsorption theory has been described by Huntsburger (Huntsberger, 1967). According to this theory, after an initial contact of the two surfaces, the material will adhere because of the surface forces acting between the atoms in the two surfaces. Weak interaction of van der Waal type plays an important role. However, if adsorption is due to chemical bonding, i.e., chemisorption, then ionic, covalent, and metallic bonds play an important role at the interface.

From a drug delivery point of view, our interest is primarily in understanding the mechanism of bioadhesion, which appears best explained by a combination of wetting, diffusion and electronic theory, although other mechanisms may be operative for a given system.

Measurement of bioadhesion

Several methods for measuring bioadhesive strength have been investigated both in vivo and in vitro, and have been reported in the literature. These techniques are thoroughly reviewed by Peppas and Buri (Peppas and Buri, 1986).

1. *In-vitro* methods.

Most in-vitro methods are based on the measurement of either shear or tensile stress (Reic and Levy, et al., 1984). One technique, reported by Smart et al. (Smart, et al., 1984), uses a Wilhelmy plate method, as shown in figure 5. In this method, the plates are coated with a polymer to be tested and immersed in a temperature controlled mucus solution. The force required to pull the plate out of the solution is determined under constant experimental conditions. Additional *in-vitro* bioadhesive tests have been described, most of which are peeling or tearing tests. The bioadhesive strength of biological cells, measured *in vitro*, have been described by Hubbe (Hubbe, 1981). Gurny (Gurny, et al., 1984) described a microbalance approach consisting of a specially designed system mounted on a typical tensile tester. This

method was used mainly to measure adhesion of a sublingual controlled release dosage form.

For purposes of drug delivery, the degree of binding of polymer to the mucin/epithelial surface is of primary importance. Robinson et al. (Park and Robinson, 1984) developed a fluorescence probe technique using cell cultures which indirectly measures the binding between a polymer and epithelial cells. Thus the binding of polymer to the lipid bilayer of a cell membrane, containing a fluorescent probe, which compresses the lipid bilayer, results in a change in fluorescence. The change in fluorescence is proportional to the binding of polymer to the cell membrane. Another method described by the same group (Park and Robinson, 1985) utilizes the force required to separate a polymer from freshly excised rabbit stomach tissue. This method uses a modified surface tensiometer and is particularly suitable for studying insoluble polymers. In this approach, a section of the tissue, as show in figure 6, having the mucus side exposed, is secured on a weighed glass vial placed in placed in a beaker containing USP simulated gastric fluid. Another section of the same tissue is placed over a rubber stopper, again with mucus side exposed and secured with a vial cap, and small quantity of polymer is placed between the two mucosal tissues. The force required to detach the polymer from the tissue is then recorded.

Another in-vitro method by Reich and this coworkers (Reich and Levy, 1984) includes the design and development of an instrument which quantitatively measures the force of detachment between the endothelium of excised rabbit cornea and polymeric material. The method of Peppas (Mikos and Peppas, 1986) utilizes a thin channel made of glass, or plexiglass, filled with artificial mucus or natural mucus maintained at 37°C. A particle of polymer is placed on the surface and subjected to a laminar flow of air with a parabolic velocity profile. By photographing the motion of the particles and determining velocities and other important parameters, both static and dynamic bioadhesive behavior were studied.

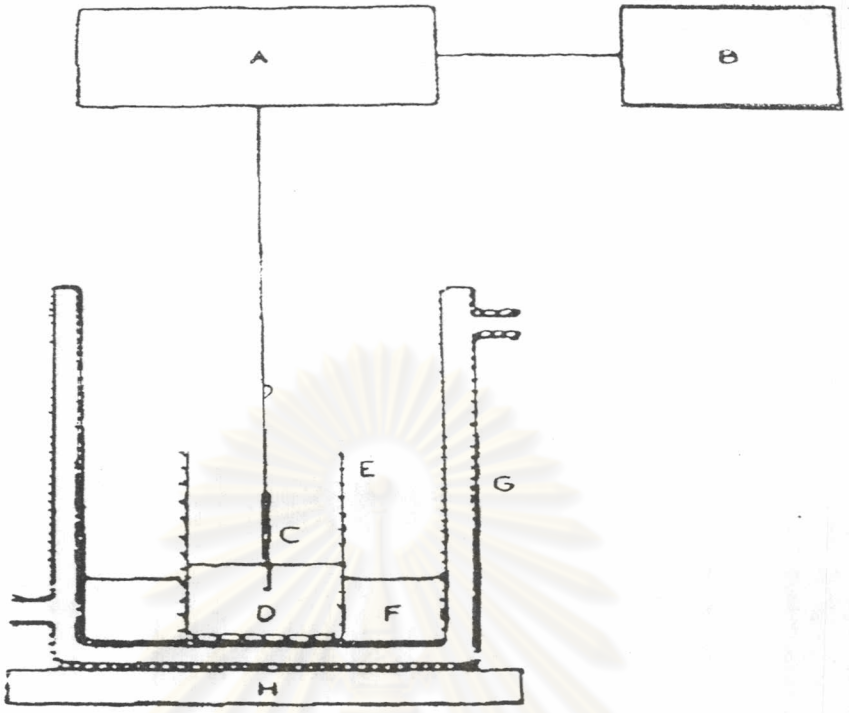

Figure 5 *In-vitro* adhesion apparatus (Smart et al., 1984)

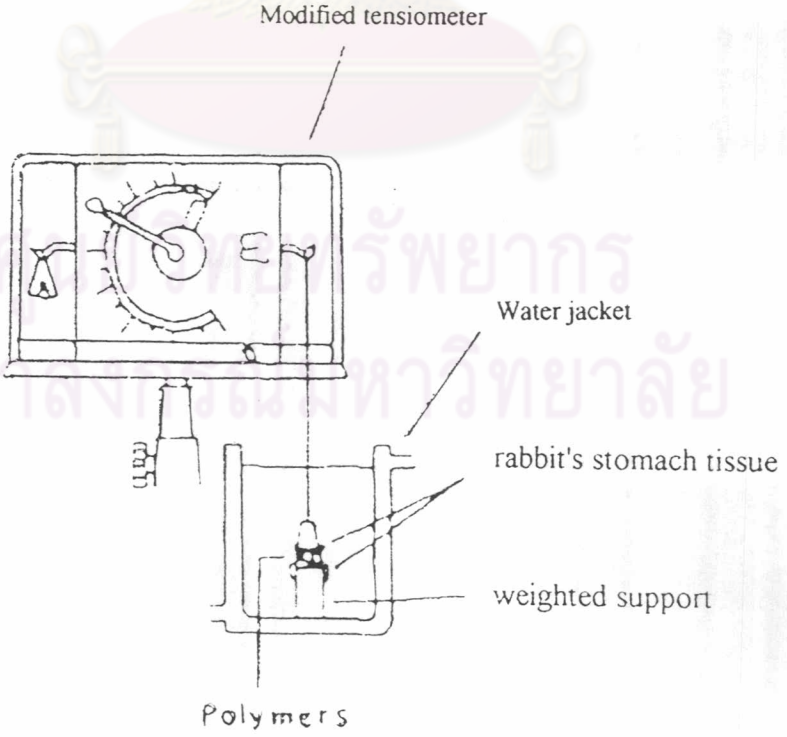

Figure 6 A modified surface tensiometer (Park and Robinson, 1985)

2. *In-vivo* methods

In-vivo methods are obviously more meaningful than in-vitro tests, because such methods presumably provide a more realistic picture of expected behavior. *In-vivo* GI transit studies, using male Sprague-Dawley rats, were conducted by administering capsules of test polymer. A capsule containing solid control or test material was surgically placed into the of anesthetized rats. The rats were permitted to awaken, and at suitable times the animals were sacrificed. Different parts of the stomach and small intestine were examined for the polymer. Davis and coworkers (Freely, et al., 1985 and Davis, 1985) have described a noninvasine technique using gamma scintigraphy for examining the bioadhesive characteristics of polymers in test animals and humans.Hunt has described a method to study the adhesive force in humans by application of the dosage form on the inner side of the lower lip.

In general, several *in-vitro* methods can be used for preliminary screening of potential candidates for adhesion. They all appear to give the same rank order dosage form containing both the bioadhesive and the drug is the best test for bioadhesion.

Formulation factors

The drug may be incorporated in the bioadhesive dosage form either by : (method 1) synthesizing the polymer with the drug in the reaction mixture and there by incorporating the drug in the matrix, or (method 2) incorporating the drug during swelling of the polymer in a saturated drug solution. One of the disadvantages of method 1 may be decomposition of the drug during polymer synthesis, particularly at high temperatures. However, with method 2 a major problem can be loading yield.

One or more adjuvant may be added to the bioadhesive dosage form depending on the nature of the drug. One of the common disadvantages of delivery via the oral mucosa is low bioavailability because of poor membrane permeability or metabolism at the absorption site. For less permeable drugs, it may be possible to add penetration enhancer to the bioadhesive system. Most penetration enhancers that are

used in transdermal delivery (Gibaldi and Feldman, 1970) have been proposed for oral delivery. Non-ionic surfactants, like polysorbates, and ionic surfactants, like sodium lauryl sulfate, have been widely used in transdermal delivery. Other agents like azone, oleic acid, ethanol, propylene glycol, bile salts, dimethyl sulfoxide, and dimethyl formamide have also been used. Extensive mechanistic studies, to understand the penetration enhancement effect by these compounds, have been reviewed by Barry (Barry, 1987). However, relatively few studies have been done in the oral cavity. Buccal permeability of insulin, dextrans, and small hydrophilic and lipophilic compounds have been show to increase by azone, ionic and non-ionic surfactants, and bile salts (Nagai, 1985, Aungst, et al., 1988, Aungst and Rogers, 1989, and Kurosaki, et al., 1989). Merkle and coworkers studied citric acid and sodium 5-methoxy salicylate as potential absorption promoters using patches of both high and low viscosity hydroxyl ethyl cellulose (HEC). The results of their studies revealed an approximately 100% increase of the mean thyrotropin concentrations with both high and low viscosity grade HEC polymers. Sodium 5-methoxy salicylate showed a similar increase with the low-viscosity polymer only. Besides conventional penetration enhancers, certain enzymes have shown promising results for buccal delivery. Squier (Squier, 1984) has reported the use of chondroitinase in increasing permeability of horseradish peroxidase without producing significant tissue damage. Tissue damage by penetration enhancers used in oral cavity bioadhesive dosage forms must be evaluated. For a penetration enhancer to be effective, it is important that its permeability action on the tissue be reversible and its toxicity during long term use be fully evaluated before use. For drugs undergoing enzymatic degradation, one can incorporate an enzyme inhibitor, particularly for peptides and proteins. Possible enzyme inhibitors have been reviewed by Junginger (Jungiger, 1990). In order to optimize the absorption of drug, the local environment may need to be modified using solubilizers or pH-modifying agents.

Bioadhesive dosage forms

Recently, a number of interesting papers dealing with adhesion have been published. With a better understanding of the mechanism of bioadhesion, several bioadhesive dosage forms have been reported. Within the oral cavity, the buccal

region has been extensively explored and appears promising for certain drugs. Hence, this region will be discussed in more depth.

1. Buccal

The buccal epithelium is highly vascularized . The papillary contour of the basal region allows efficient vascularization of the cell layer. The higher permeability of this tissue, in comparison with skin, effective vascularization, bypass of first pass metabolism, and accessibility of this tissue represents advantages in delivery of therapeutic agents via the buccal route. Because of the presence of a smooth and relatively immobile surface for placement of a bioadhesive dosage form, the buccal region appears to be more suitable for sustained delivery of therapeutic agents using a bioadhesive system.

Particularly with peptide drugs, low permeability of the tissue and enzymatic degradation are two major factors for low bioavailability. The buccal membrane is reported to lack surface-bound peptidase and carbohydrases (Giannitsis, et al., 1972). Using 4-methoxy-2-naphthyl amides of leucine and alanine as substrates, Stratford (Stratfordand, 1986) has shown that in albino rabbits, the buccai mucosal activity of amino peptidase, using tissue homogenates, is comparable to ileal and duodenal amino peptidase. Aminopeptidase N and A (plasma membrane bound peptidase), and aminopeptidase B (cytosolic enzymes) were found in the buccal tissue (Kashi and Lee, 1986). However, the enzymatic activity using this method may be overestimated, and further work is required to establish the role of subcellular organization of these aminopeptidases, which was disrupted using this methodology. Studies with insulin and proinsulin showed buccal protease activity to be 4-times less compared to the gut. However, in all these studies, no attempts were made to distinguish between extracellular and intracellular activity of the mucosa. Schurr and coworkers (Schurr, et al., 1985) have attempted to deliver peptides like thyrotropin releasing hormone by designing a self adhesive buccal patch. A more prolonged TRH effect was observed after buccal administration, compared to nasal and intravenous administration. However, the blood levels were not close to the therapeutically desired levels. Particularly with peptide drugs, low permeability of the tissue and enzymatic degradation are two major factors for low bioavailability. Both the issues

of poor permeation and enzymatic degradation for peptide delivery can be overcome by incorporation of penetration enhancers and suitable enzyme inhibitors in the bioadhesive mucosal dosage form. Since there is a limit to the size of the bioadhesive dosage form, only a limited amount of drug can be used in these systems. In general, any drug with a daily requirement of 25 mg or less would be a candidate for buccal delivery. Drugs with short biological half-lives requiring a sustained effect, poor permeability, sensitivity to enzymatic degradation, and poor solubility may be successfully delivered via a bioadhesive oral mucosal delivery system. Relevant bioadhesive dosage forms in the buccal cavity include adhesive tablets, adhesive gels, adhesive patches, and adhesive ointments. These dosage forms will be discussed briefly.

1.1 Adhesive tablets

Unlike conventional tablets bioadhesive tablets allow drinking and speaking without major discomfort. Triamcinolone acetonide (Nagai and Machida, 1985) has been formulated using the principles of bioadhesion for the treatment of aphthous stomatitis. The dosage form is a small, thin, double layered tablet, as shown in figure 7. The upper supporting layer consists of lactose, and the lower layer has a bioadhesive polymer, hydroxypropylcellulose or carbomer, This formulation was effective at a lower dose than ointment. It is currently marketed in Japan under the trade name Aftach®. Davis (Davis, et al., 1982) developed adhesive tablets on the basis of eroding/hydrocolloid filler tablets. The tablets remained in place for about 3 hours, as shown by scintigraphy. Schor (Schor, et al., 1983) developed nitroglycerin bioadhesive tablets, Susadrin or Suscal for angina pectoris.

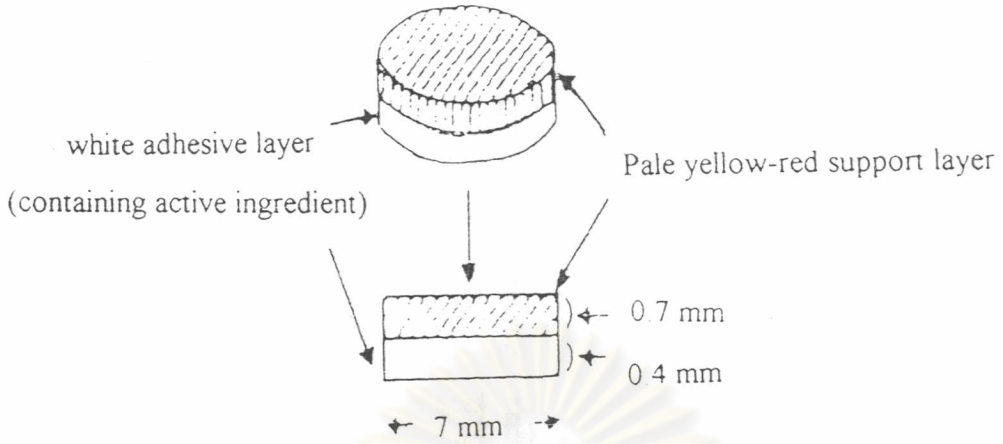

Figure 7 Adhesive tablet (Nagai and Machida, 1985)

1.2 Adhesive gels

Various adhesive gels may be used to deliver drugs via the buccal mucosa and allow sustained release. In comparison to solutions, gels can significantly prolong residence time and hence improve bioavailability. Gels can be used for local therapy, as suggested in the work by Ishida et al. (Ishida, et al., 1982 and Ishida, et al., 1983). Polyacrylic acid and polymethylmethacrylate have been used as gel forming polymers.

1.3 Adhesive patches

These are the most extensively studied dosage forms for oral drug delivery. Patches may range from simple erodible or nonerodible adhesive disks to laminated systems (Ishida, et al., 1981, and Anders and Merkel, 1989). The size of the patches can vary from 1 to 15 cm^2. The smaller the size, the more convenient and comfortable are the patches. Patches may be formulated with a backing layer providing unidirectional release of the drug into the mucus layer, thus minimizing loss of drug to the saliva and maximizing concentration gradient of the drug to the mucosa. On the other hand, the adhesive polymer may be used for local drug release, with no backing layer. Such patches will provide a bi-directinal release of drug, resulting in significant loss during swallowing of saliva. Anders (Anders and Merkel,

1989) designed a bilayer patch (polytef-disk) consisting of proliterin for thyroid gland diagnosis. This patch has a backing layer of teflon and mucoadhesive layer of proliterin dispersed in hydroxyethylcellulose. Velliard (Veillard, et al., 1987) reported the use of unidirectional buccal patch as shown in Figure 8. It consisted of three layers: (a) an impermeable backing; (b) a rate limiting center membrane containing the drug; (c) a mucoadhesive basement layer containing biadhesive polymer polycarbophil.

The bioadhesive polymer swells, creating a flexible network through which diffusion of drug takes place. This patch has been tested in dog buccal mucosa and was shown to remain in place for up to 17 hours without any obvious discomfort, irrespective of food or drink consumed. This patch showed similar results when tested in humans.

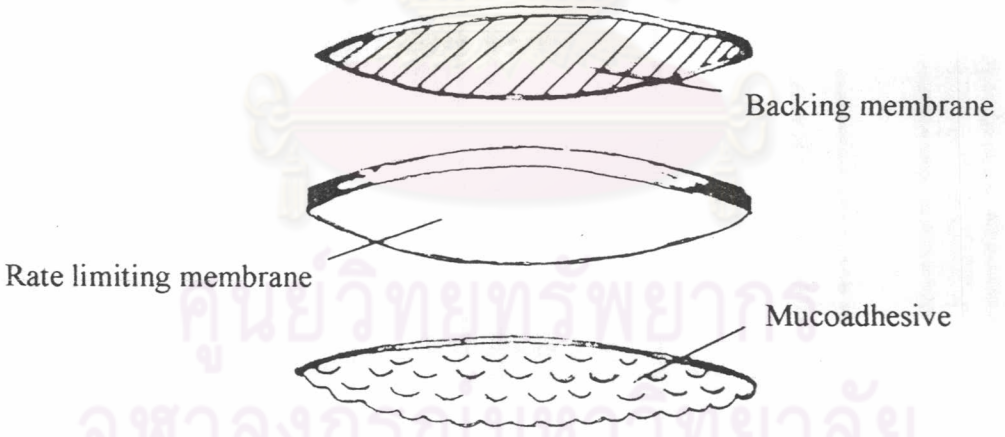

Figure 8 Proposed design of unidirectional patch

1.4 Adhesive ointments

Ishida et al. (Ishida, et al., 1983) investigated the availability of steroid from an oral ointment base containing carbopol-934 from hamster's cheek pouch. The local drug activity for treatment of aphtha was investigated using prednisolone as a model drug, Three bases white petrolatum, hydrophilic petrolatum, and lauromacrogol along with carbopol was incorporate in the ointment. The release of prednisolone was not observed in an ointment containing hydrophilic petrolatum and carbopol, since the ointment was not gelled due to its poor wettability and high consistency. Bioadhesive ointments have not been investigated as extensively as tablets and patches.

1.5 Sublingual

The sublingual region generally shows higher drug permeability than the buccal region. However, unlike the buccal region, the sublingual region does not appear promising for attachment of a bioadhesive system, primarily because of the physical structure and mobility of tissue in this area. Gurny, et al. (Gurny, et al., 1984), using a bioadhesive paste, attempted to deliver febuverine sublingually. They used 20% sodium carboxy methylcellulose, 14% hydrolyzed gelatin, and PEG gel making up the rest of the formulation. This route has been used extensively for delivery of drugs which require a rapid onset of action, e.g.,nitroglycerin.

1.6 Dental/gingival

Work on adhesive materials and evaluation techniques, relative to denture adhesives, have been extensively reviewed by Ali (Ali, 1988). Denture adhesives, as defined by Ali, are devices that are prescribed as an aid to retain dentures or reduce discomfort after the insertion of dentures. Both natural and synthetic hydrocolloids have been used for denture adhesives. The excipients of denture adhesives include swellable polymers, gel, antibacterial/antiseptic agents, preservatives, fillers, wetting, and flavoring agents. Rosenthal has published a listing of denture adhesive combinations containing both natural and synthetic hydrocolloids or combinations. The disadvantages of using denture adhesives are the short and variable duration of

action, nausea, damage to the prosthesis, and the danger of prolonging the service life of an ill-fitting denture.

Gingival plasters containing $PGF_{2\alpha}$ and PGE_2 were formulated by Nagai et al. (Nagai, et al., 1990) to provide a continuous, slow release of prostaglandin into the gingival tissues for orthodontic tooth movement. The formulation consisted of a backing layer to limit the release of prostaglandin into the mouth and the active ingredient was incorporated into the water-activated adhesive layer. The major components in the $PGF_{2\alpha}$ formulation were synthetic resin, natural gum, hydrophobic polymer, polyethylene glycol, glycerin, agar, castor oil, and other excipients. The system did not present any irritation problems when tested *in vivo*. Such a plaster-type gingival therapeutic system appears promising for gingival delivery of drugs, or for a local effect in the mouth. Another dosage form studied for gingival delivery is a bioadhesive dosage form containing lidocaine developed by Ishida et al. (Ishida, et al., 1982). This was an adhesive tablet dosage form containing magnesium stearate in the cap layer and HPC and PC in the base. These tablets were tested in humans. The dosage form was expected to afford a prolonged anesthetic action for the treatment of toothache with very rapid onset of action and lasting approximately 4 hours without anesthetizing other parts of the oral cavity.

Mechanical Properties of film (Aulton and Abdul-Razzak, 1981)

The elasticity and tensile strength of the various films can be evaluated by using a tensile-strength tester. The tensile testing process is to apply increasing tensile load at a constant rate to a film strip which know dimensions in the dimension perpendicular to cross-section of film strip until the failure takes place. The load at film failure will be measured in term of force per unit cross-section area of the film.

Polymers are divided into five categories according to qualitative description of their mechanical behavior corresponding stress-strain characteristics as showed in the table 1 and figure 9A.

Table 1 Qualitative description of polymer and it's stress-strain characteristics

Polymer Description	Characteristics of stress-strain curve			
	Young's Modulus	Yield Stress	Tensile Strength	Elongation to Break
Soft, weak	Low	Low	Low	Low to moderate
Soft, tough	Low	Low	Moderate	Very high (20-100%)
Hard, brittle	High	none	Moderate to high	Very low (<2%)
Hard, strong	High	High	High	Moderate (~5%)
Hard, tough	High	High	High	High

Hard or stiff polymers are characterized by high modulus as opposed to soft ones. Strong (as opposed to weak) polymers have high tensile strengths. Tough (as opposed to brittle) polymers have large area under their stress-strain curves and require large amounts of energy to break under stress, combining high or at least moderate tensile strength with high elongation. The desirable hard, tough film must have a high yield stress large extension before breaking and high elastic modulus.

A typical stress-strain curve is shown in figure 9B. The ultimate tensile strength or breaking stress is the maximum applied at which the film breaks. Stress is calculated by dividing force by original cross-sectional area. Elongation or strain at break is a measure of the ductility of the films. Strain in tension called elongation. It is calculated by diving the increase in length by original length. Elastic modulus or Young's modulus is the most basic and structurally important of all mechanical properties and is a measure of the stiffness and rigidity of the film. It is calculated as applied stress divided by the corresponding strain in the region of linear elasticity. Area under curve is a function of the work done in breaking the film and is representative of the film's toughness. The energy to break per unit area is calculated by diving the area under curve by the volume of the specimen between the clamps.

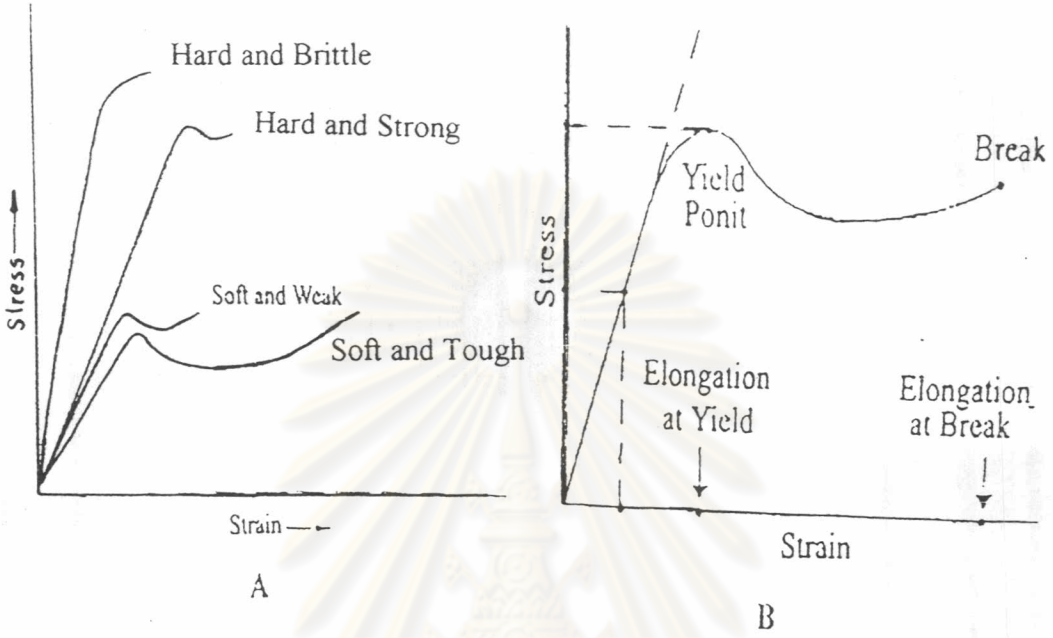

Figure 9 Stress-strain curve: (A) characteristic of polymer properties in stress-strain curves; (B) typical stress-strain curve