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# Critical Review on Mucoadhesive Drug Delivery Systems

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## Abstract

***Plan**: This review considers the basic mechanisms by which mucoadhesive can adhere to a mucous membrane in terms of the nature of the adhering surfaces and the forces that may be generated to secure them together. Mucosal adhesion is backed by several theories which include electronic, adsorption, wetting, diffusion, fracture and mechanical. Stages of mucoadhesion include contact stage and consolidation stage.*

***Prologue**: Much attention has been focused in the field of mucoadhesive formulation developments compared to other delivery systems. Mucoadhesion while considering drug delivery is having several merits, because of the ideal physiochemical characters of the mucosal membrane. Ideally a mucoadhesive dosage form interacts with the mucosal membrane by ionic bonds, covalent bonds, Van-der-Waal bonds and hydrogen bonds. Various sites for mucoadhesive drug delivery system are ocular, nasal, buccal cavity; GIT, vaginal, rectal and several specific dosage forms have been reported. Factors affecting mucoadhesion are molecular weight, flexibility of polymer chain, pH, presence of carboxylate group and density. Several synthetic and natural polymers are identified as suitable candidates for mucoadhesive formulation. Ex-vivo/in-vitro studies utilizing gut sac of rats provides in-depth knowledge about the adhesive property of the dosage form as well as polymers. AFM can be used as a part of imaging methods.*

***Outcome**: Mucoadhesive drug delivery system shows promising future in enhancing the bioavailability and specific needs by utilizing the physiochemical characters of both the dosage form and the mucosal lining. It has to be noted that only a moist surface can bring the mucoadhesive nature of the dosage form.*

***Key Words:** Bioadhesion, mucoadhesion, Van-der-Waal force, consolidation stage.*

## 1. Introduction

Bioadhesion may be defined as the state in which two materials, at least one of which is biological in nature, are held together for extended period of time by interfacial forces. In pharmaceutical sciences, when the adhesive attachment is to mucus or a mucous membrane, the phenomenon is referred to as mucoadhesion[1].In the early 1980s; academic research groups working in the ophthalmic field pioneered the concept of mucoadhesion as a new strategy to improve the efficacy of various drug delivery systems. Since then, the potential of mucoadhesive polymers was shown in ocular, nasal, vagina and buccal drug delivery systems leading to a significantly prolonged residence time of sustained release delivery systems on this mucosal membranes[2-5]. In addition, the development of oral mucoadhesive delivery systems was always of great interest as delivery systems capable of adhering to certain gastrointestinal (GI) segments would offer various advantages.

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With few exceptions, however, mucoadhesive drug delivery systems have so far not reached their full potential in oral drug delivery, because the adhesion of drug delivery systems in the GI tract is in most cases insufficient to provide a prolonged residence time of delivery systems in the stomach or small intestine[6-8].

The need to deliver 'challenging' molecules such as biopharmaceuticals (proteins and oligonucleotides) has increased interest in this area. Mucoadhesive materials could also be used as therapeutic agents in their own right, to coat and protect damaged tissues (gastric ulcers or lesions of the oral mucosa) or to act as lubricating agents (in the oral cavity, eye and vagina).

*Mucous Membranes*

Mucous membranes (mucosae) are the moist surfaces, lining the walls of various body cavities such as the gastrointestinal and respiratory tracts. They consist of a connective tissue layer (the lamina propria) above which is an epithelial layer, the surface of which is made moist usually by the presence of a mucus layer. The epithelia may be either single layered (e.g. the stomach, small and large intestine and bronchi) or multilayered/stratified (e.g. in the oesophagus, vagina and cornea). The former contain goblet cells which secrete mucus directly onto the epithelial surfaces, the latter contain, or are adjacent to tissues containing, specialized glands such as salivary glands that secrete mucus onto the epithelial surface. Mucus is present as either a gel layer adherent to the mucosal surface or as a luminal soluble or suspended form. The major components of all mucus gels are mucin glycoproteins, lipids, inorganic salts and water, the latter accounting for more than 95% of its weight, making it a highly hydrated system. The mucin glycoproteins are the most important structure-forming component of the mucus gel, resulting in its characteristic gel-like, cohesive and adhesive properties. The thickness of this mucus layer varies on different mucosal surfaces, from 50 to 450 µm in the stomach, to less than 1 µm in the oral cavity. The major functions of mucus are that of protection and lubrication (they could be said to act as anti-adherents)[9-12].

Other than the low surface area available for drug absorption in the buccal cavity, the retention of the dosage format the site of absorption is another factor which determines the success or failure of buccal drug delivery system. The utilization of mucoadhesive systems is essential to maintain an intimate and prolonged contact of the formulation with the oral mucosa allowing a longer duration for absorption. Some adhesive systems deliver the drug towards the mucosa only with an impermeable product surface exposed to the oral cavity which prevents the drug release into oral cavity. For example, Lopez and co-workers designed bilaminated films to provide unidirectional release of drug and avoid buccal leakage. They contained a bioadhesive layer made up of chitosan, polycarbophil, sodium alginate and gellan gum while backing layer made up of ethyl cellulose.

*Composition of Mucus Layer*

Mucus is translucent and viscid secretion which forms a thin, continuous gel blanket adherent to the mucosal epithelial surface[13].

Mucus glycoprotiens are high molecular proteins possessing attached oligosaccharide units containing the composition of mucus is given in table no 1.

a) L-fucose
b) D-galactose
c) N-acetyl-D-glucosamine
d) N-acetyl-D-galactosamine
e) Sialic acid

*Sites for mucoadhesive drug delivery systems*

The common sites of application where mucoadhesive drug delivery systems have the ability to delivery pharmacologically active agents include oral cavity, eye conjunctiva, vagina, nasal cavity and gastrointestinal tract. The current section of the review will give an overview of the above-mentioned delivery sites.

The buccal cavity has a very limited surface area of around 50 $cm^2$ but the easy access to the site makes it a preferred location for delivering active agents. The site provides an opportunity to deliver pharmacologically active agents systemically by avoiding hepatic first-pass metabolism in addition to the local treatment of the oral lesions. The sublingual mucosa is relatively more permeable than the buccal mucosa (due to the presence of large number of smooth muscle and immobile mucosa), hence formulations for sublingual delivery are designed to release the active agent quickly while mucoadhesive formulation is of importance for the delivery of active agents to the buccal mucosa where the active agent has to be released in a controlled manner. This makes the buccal cavity more suitable for mucoadhesive drug delivery[14].

Like buccal cavity, nasal cavity also provides a potential site for the development of formulations where mucoadhesive polymers can play an important role. The nasal mucosal layer has a surface area of around 150-200 $cm^2$. The residence time of a particulate matter in the nasal mucosa varies between 15 and 30 min, which have been attributed to the increased activity of the mucociliary layer in the presence of foreign particulate matter[15].

Ophthalmic mucoadhesives also is another area of interest. Due to the continuous formation of tears and blinking of eye lids there is a rapid removal of the active medicament from the ocular cavity, which results in the poor bioavailability of the active agents. This can be minimized by delivering the drugs using ocular insert or patches[16-18].

The vaginal and the rectal lumen have also been explored for the delivery of the active agents both systemically and locally. The active agents meant for the systemic delivery by this route of administration bypasses the hepatic first-pass metabolism. Quite often the delivery systems suffer from migration within the vaginal/rectal lumen which might affect the delivery of the active agent to the specific location[19-21].

Gastrointestinal tract is also a potential site which has been explored since long for the development of mucoadhesive based formulations. The modulation of the transit time of the delivery systems in a particular location of the gastrointestinal system by using mucoadhesive polymers has generated much interest among researchers around the world[22].

*The mucoadhesive / mucosa interaction*

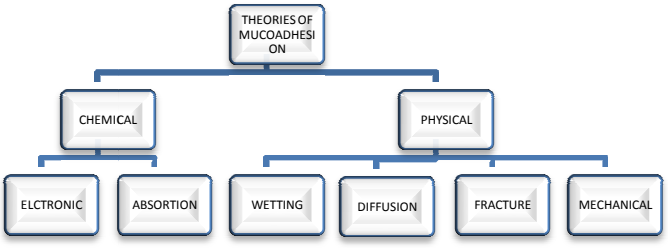
A. *Chemical bonds*

For adhesion to occur, molecules must bond across the interface. These bonds can arise in the following way[23].

- Ionic bonds—where two oppositely charged ions attract each other via electrostatic interactions to form a strong bond (e.g. in a salt crystal).
- Covalent bonds—where electrons are shared, in pairs, between the bonded atoms in order to 'fill' the orbitals in both. These are also strong bonds.
- Hydrogen bonds—here a hydrogen atom, when covalently bonded to electronegative atoms such as oxygen, fluorine or nitrogen, carries a slight positively charge and is therefore is attracted to other electronegative atoms. The hydrogen can therefore be thought of as being shared, and the bond formed is generally weaker than ionic or covalent bonds.
- Van-der-Waals bonds—these are some of the weakest forms of interaction that arise from dipole–dipole and dipole-induced dipole attractions in polar molecules, and dispersion forces with non-polar substances.
- Hydrophobic bonds—more accurately described as the hydrophobic effect, these are indirect bonds (such groups only appear to be attracted to each other) that occur when non-polar groups are present in an aqueous solution. Water molecules adjacent to non-polar groups form hydrogen bonded structures, which lowers the system entropy. There is therefore an increase in the tendency of non-polar groups to associate with each other to minimize this effect. Progression of bond receptor is explained diagrammatically in fig 2.

B. *Mucoadhesion Theories*

It is reported that, although the chemical and physical basis of mucoadhesion are not yet well understood, there are six classical theories adapted from studies on the performance of several materials and polymer-polymer adhesion which explain the phenomenon. Contact angle and time plays a major role in mucoadhesion.



*Electronic theory*

Electronic theory is based on the premise that both mucoadhesive and biological materials possess opposing electrical charges. Thus, when both materials come into contact, they transfer electrons leading to the building of a double electronic layer at the interface, where the attractive forces within this electronic double layer determines the mucoadhesive strength.

1. *Adsorption theory*

According to the adsorption theory, the mucoadhesive device adheres to the mucus by secondary chemical interactions, such as in Van der Waals and hydrogen bonds, electrostatic attraction or hydrophobic interactions. For example, hydrogen bonds are the prevalent interfacial forces in polymers containing carboxyl groups. Such forces have been considered the most important in the adhesive interaction phenomenon because, although they are individually weak, a great number of interactions can result in an intense global adhesion.

2. *Wetting theory*

The wetting theory applies to liquid systems which present affinity to the surface in order to spread over it. This affinity can be found by using measuring techniques such as the contact angle. The general rule states that the lower the contact angle then the greater the affinity (Figure 1). The contact angle should be equal or close to zero to provide adequate spreadability.

3. *Diffusion theory*

Diffusion theory describes the interpenetration of both polymer and mucin chains to a sufficient depth to create a semi-permanent adhesive bond. It is believed that the adhesion force increases with the degree of penetration of the polymer chains. This penetration rate depends on the diffusion coefficient, flexibility and nature of the mucoadhesive chains, mobility and contact time. The adhesion strength for a polymer is reached when the depth of penetration is approximately equivalent to the polymer chain size. In order for diffusion to occur, it is important that the components involved have good mutual solubility, that is, both the bioadhesive and the mucus have similar chemical structures. The greater the structural similarity, the better the mucoadhesive bond.

4. *Fracture theory*

This is perhaps the most-used theory in studies on the mechanical measurement of mucoadhesion. It analyses the force required to separate two surfaces after adhesion is established (Figure 2). This force, $s_m$, is frequently calculated in tests of resistance to rupture by the ratio of the maximal detachment force, $F_m$, and the total surface area, $A_0$, involved in the adhesive interaction (equation 1):

$$S_m = \frac{F_m}{A_0} \quad \text{..... (1)}$$

Since the fracture theory is concerned only with the force required to separate the parts, it does not take into account the interpenetration or diffusion of polymer chains.

Consequently, it is appropriate for use in the calculations for rigid or semi-rigid bioadhesive materials, in which the polymer chains do not penetrate into the mucus layer.

5. *Mechanical theory*

Mechanical theory considers adhesion to be due to the filling of the irregularities on a rough surface by a mucoadhesive liquid. Moreover, such roughness increases the interfacial area available to interactions thereby aiding dissipating energy and can be considered the most important phenomenon of the process.

Lee, Park, Robinson, 2000 had described that it is unlikely that the mucoadhesion process is the same for all cases and therefore it cannot be described by a single theory. In fact, all theories are relevant to identify the important process variables.

The mechanisms governing mucoadhesion are also determined by the intrinsic properties of the formulation and by the environment in which it is applied. Intrinsic factors of the polymer are related to its molecular weight, concentration and chain flexibility. For linear polymers, mucoadhesion increases with molecular weight, but the same relationship does not hold for non-linear polymers. It has been shown that more concentrated mucoadhesive dispersions are retained on the mucous membrane for longer periods, as in the case of systems formed by *in situ* gelification. After application, such systems spread easily, since they present rheological properties of a liquid, but gelify as they come into contact the absorption site, thus preventing their rapid removal. Chain flexibility is critical to consolidate the interpenetration between formulation and mucus.

Environment-related factors include pH, initial contact time, swelling and physiological variations. The pH can influence the formation of ionizable groups in polymers as well as the formation of charges on the mucus surface. Contact time between mucoadhesive and mucus layer determines the extent of chain interpenetration. Super-hydration of the system can lead to build up of mucilage without adhesion. The thickness of the mucus layer can vary from 50 to 450 μm in the stomach to less than 1μm in the oral cavity. Other physiological variations can also occur with diseases.

C. *Mechanisms of Mucoadhesion*

The mucoadhesive must spread over the substrate to initiate close contact and increase surface contact, promoting the diffusion of its chains within the mucus. Attraction and repulsion forces arise and, for a mucoadhesive to be successful, the attraction forces must dominate.

Each step can be facilitated by the nature of the dosage form and how it is administered. For example, a partially hydrated polymer can be adsorbed by the substrate because of the attraction by the surface water[24].

Due to its relative complexity, it is likely that the process of mucoadhesion cannot be described by just one of these theories. Lee, Park, Robinson, 2000 had described the mechanism of mucoadhesion in four different approaches (Figure 3). These include:

- Dry or partially hydrated dosage forms contacting surfaces with substantial mucus layers (typically particulates administered into the nasal cavity).
- Fully hydrated dosage forms contacting surfaces with substantial mucus layers (typically particulates of many mucoadhesive that have hydrated in the luminal contents on delivery to the lower gastrointestinal tract).
- Dry or partially hydrated dosage forms contacting surfaces with thin/discontinuous mucus layers (typically tablets or patches in the oral cavity or vagina).
- Fully hydrated dosage forms contacting surfaces with thin/discontinuous mucus layers (typically aqueous semisolids or liquids administered into the esophagus or eye).

It is unlikely that the mucoadhesive process will be the same in each case. In the study of adhesion generally, two stages in the adhesive process supports the mechanism of interaction between mucoadhesive materials and a mucous membrane Thus, the mechanism of mucoadhesion is generally divided in two stages, the contact stage and the consolidation stage.
*Stage 1* —Contact stage: An intimate contact (wetting) occurs between the mucoadhesive and mucous membrane.
*Stage 2* —Consolidation stage: Various physicochemical interactions occur to consolidate and strengthen the adhesive joint, leading to prolonged adhesion.

In some cases, such as for ocular or vaginal formulations, the delivery system is mechanically attached over the membrane. In other cases, the deposition is promoted by the aerodynamics of the organ to which the system is administered, such as for the nasal route. On the other hand, in the gastrointestinal tract direct formulation attachment over the mucous membrane is not feasible. Peristaltic motions can contribute to this contact, but there is little evidence in the literature showing appropriate adhesion. Additionally, an undesirable adhesion in the esophagus can occur. In these cases, mucoadhesion can be explained by peristalsis, the motion of organic fluids in the organ cavity, or by Brownian motion. If the particle approaches the mucous surface, it will come into contact with repulsive forces (osmotic pressure, electrostatic repulsion, etc.) and attractive forces (van der Waals forces and electrostatic attraction). Therefore, the particle must overcome this repulsive barrier[26].

In the consolidation step, the mucoadhesive materials are activated by the presence of moisture. Moisture plasticizes the system, allowing the mucoadhesive molecules to break free and to link up by weak van der Waals and hydrogen bonds. Essentially, there are two theories explaining the consolidation step: the diffusion theory and the dehydration theory.

According to diffusion theory, the mucoadhesive molecules and the glycoproteins of the mucus mutually interact by means of interpenetration of their chains and the building of secondary bonds (Smart, 2005). For this to take place the mucoadhesive device has features favoring both chemical and mechanical interactions.

For example, molecules with hydrogen bonds building groups (–OH, –COOH), with an anionic surface charge, high molecular weight, flexible chains and surface-active properties, which induct its spread throughout the mucus layer, can present mucoadhesive properties[26].

According to dehydration theory (Figure 4), materials that are able to readily gelify in an aqueous environment, when placed in contact with the mucus can cause its dehydration due to the difference of osmotic pressure. The difference in concentration gradient draws the water into the formulation until the osmotic balance is reached.

This process leads to the mixture of formulation and mucus and can thus increase contact time with the mucous membrane. Therefore, it is the water motion that leads to the consolidation of the adhesive bond, and not the interpenetration of macromolecular chains. However, the dehydration theory is not applicable for solid formulations or highly hydrated forms.

*Factors Affecting Mucoadhesion*

Several factors have been identified as affecting the strength of the solid mucoadhesive joint. Many studies have indicated an optimum molecular weight for mucoadhesion, ranging from circa $10^4$ Da to circa $4\times10^6$ Da, although accurately characterizing the molecular weight of large hydrophilic polymers is very difficult. Larger molecular weight polymers will not hydrate readily to free the binding groups to interact with a substrate, while lower molecular weight polymers will form weak gels and readily dissolve. The flexibility of polymer chains is believed to be important for interpenetration and entanglement, allowing binding groups to come together. As the cross-linking of water-soluble polymers increases, the mobility of the polymer chains decrease, although this could also have a positive effect in restricting over hydration. Studies have shown that the mucoadhesive properties of polymers containing ionisable groups are affected by the pH of the surrounding media. For example, mucoadhesion of poly(acrylicacid)s is favoured when the majority of the carboxylate groups are in the unionised form, which occurs at pHs below the pKa. However, in systems with a high density of ionisable groups (e.g. carbomers or chitosans), the local pH within or at the surface of a formulation will differ significantly from that of the surrounding environment.

The strength of adhesion has been found to change with the initial ‘consolidation’ force applied to the joint, or the length of contact time prior to testing. The presence of metal ions, which can interact with charged polymers, may also affect the adhesion process[27-28].

*Mucoadhesive Polymers*

Mucoadhesive polymers are water-soluble and water insoluble polymers, which are swellable networks, jointed by cross-linking agents. These polymers possess optimal polarity to make sure that they permit sufficient wetting by the mucus and optimal fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place.
Mucoadhesive polymers that adhere to the mucin-epithelial surface can be conveniently divided into three broad classes:

- Polymers that become sticky when placed in water and owe their mucoadhesion to stickiness.
- Polymers that adhere through nonspecific, non-covalent interactions that is primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant).
- Polymers that bind to specific receptor site on tile self surface.

*A. Characteristics of an ideal mucoadhesive polymer*

*An ideal mucoadhesive polymer has the following characteristics[29-30]:*

- The polymer and its degradation products should be nontoxic and should be non-absorbable from the gastrointestinal tract.
- It should be nonirritant to the mucous membrane.
- It should preferably form a strong non-covalent bond with the mucin-epithelial cell surfaces.
- It should adhere quickly to most tissue and should possess some site-specificity.
- It should allow daily incorporation to the drug and offer no hindrance to its release.
- The polymer must not decompose on storage or during the shelf life of the dosage form.
- The cost of polymer should not be high so that the prepared dosage form remains competitive.

*B. Molecular characteristics*

*The properties exhibited by a good mucoadhesive may be summarized as follows[31-32]:*

- Strong hydrogen bonding groups (-OH, -COOH).
- Strong anionic charges.
- Sufficient flexibility to penetrate the mucus network or tissue crevices.
- Surface tension characteristics suitable for wetting mucus/mucosal tissue surface.
- High molecular weight.

Although an anionic nature is preferable for a good mucoadhesive, a range of nonionic molecules (e.g., cellulose derivatives) and some cationic (e.g., Chitosan) can be successfully used.

A short list of mucoadhesive polymers is given below:

*Synthetic polymers:*

*Cellulose derivatives (methylcellulose, ethyl cellulose, hydroxy-ethylcellulose, Hydroxyl propyl cellulose, hydroxyl propyl methylcellulose, sodium carboxy methylcellulose, Poly (acrylic acid) polymers (carbomers, polycarbophil), Poly (hydroxyethyl methylacrylate), Poly (ethylene oxide), Poly (vinyl pyrrolidone), Poly (vinyl alcohol).*

*1. Natural polymers:*

Tragacanth, Sodium alginate, Karaya gum, Guar gum, Xanthan gum, Lectin, Soluble starch, Gelatin, Pectin, Chitosan.

*New generation of mucoadhesive polymers*

In a recent mini-review by Lee et al. current bioadhesive polymers are classified as first generation and second generation. The older generation of mucoadhesive polymers, referred to as off-the shelf polymers, lack specificity and targeting capability. They adhere to the mucus non-specifically, and suffer short retention times due to the turnover rate of the mucus. The chemical interactions between mucoadhesive polymers and the mucus or tissue surfaces are generally non-covalent in nature, and are classified as consisting mostly of hydrogen bonds, hydrophobic, and electrostatic interactions. However, newer polymers are capable of forming covalent bonds with the mucus and the underlying cell layers, and hence, exhibit improved chemical interactions.

The new generation of mucoadhesives (with the exception of thiolated polymers) can adhere directly to the cell surface, rather than to mucus. They interact with the cell surface by means of specific receptors or covalent bonding instead of non-specific mechanisms, which are characteristic of the previous polymers. We have chosen to focus on recently discovered bioadhesive polymers in this review. Examples of such are the incorporation of l-cysteine into thiolated polymers and the target-specific, lectinmediated adhesive polymers. These classes of polymers hold promise for the delivery of a wide variety of new drug molecules, particularly macromolecules, and create new possibilities for more specific drug–receptor interactions and improved targeted drug delivery.

*Thiolated mucoadhesive polymers*

Through a covalent attachment between a cysteine (Cys) residue and a polymer of choice, such as polycarbophil, poly(acrylic acid), and chitosan, a new generation of mucoadhesive polymers have been created. The modified polymers, which contain a carbodiimide-mediated thiol bond, exhibit much-improved bioadhesive properties.

Investigations of the GI epithelial mucus have clarified the structure of this gel-like biopolymer. With more than 4500 amino acids, the enormous polypeptide backbone of mucin protein is divided into three major subunits; tandem repeat array, carboxyl and amino-terminal domains. The carboxyl-terminal domain contains more than 10% of cysteine residues. The amino-terminal domain also contains Cys-rich regions.

The Cys-rich sub-domains are responsible for forming the large oligomers of mucin through disulfide bonds. Based on the disulfide exchange reaction, disulfide bonds between the mucin glycoprotein and the thiolated mucoadhesive polymer can potentially be formed, which results in a strong covalent interaction. Other improved mucoadhesive properties of the thiolated polymers, such as improved tensile strength, high cohesive properties, rapid swelling, and water uptake behavior, have made them an attractive new generation of bioadhesive polymers.

As one example to illustrate the improved bioadhesive properties of thiolated polymers, Bernkop-Schnurch et al. have reported a positive correlation between the adhesive properties and increasing amounts of the polymer in dry compacts of polycarbophil covalently bound to cysteine.

Recently, a model pentapeptide (Leu-enkephalin)was successfully delivered via the buccal mucosa, taking advantage of the improved adhesion time due to the specific interaction of a polycarbophil–cysteine conjugated (thiolated) polymer with the buccal mucosa, as well as its enzyme inhibitory effect (see the enzyme inhibitors section).

*Target-specific, lectin-mediated bioadhesive polymers*

The possibility of developing a bioadhesive polymer which is able to selectively create specific molecular interactions with a particular target, such as a receptor on the cell membrane of a specific tissue, is a very attractive potential for targeted delivery. The potential of a specific receptor–bioadhesive polymer interaction can circumvent the limiting factors of rapid mucus turnover and short residence time. Unlike general mucoadhesive polymers, which bind to the mucosal surface ubiquitously, a specific receptor mediated interaction with the mucosal surface could allow for direct binding to the cell surface, rather than only the mucus layer. Specific proteins or glycoproteins, such as lectins, which are able to bind certain sugars on the cell membrane, can increase bioadhesion and potentially improve drug delivery via specific binding and increase the residence time of the dosage form. This type of bioadhesion should be more appropriately termed as cytoadhesion. A site-specific interaction with the receptor could potentially trigger intercellular signaling for internalization of the drug or the carrier system (endocytosis through cytoadhesion) into the lysosomes or into other cellular compartments.

Although lectins are also found in bacteria, those from the plant kingdom still remain the largest group of this class. Lectin isolated from tomato fruit (Lycopersicum esculentum) has been reported to specifically and safely bind N-acetylglucosamine (Glu-NAc) on the surface of several cell monolayers. Woodley and Naisbett demonstrated the application of tomato lectin (TL) in oral drug delivery for the first time. It has been shown that TL can bind rat intestinal epithelium safely without inducing any harmful effects on the membrane. Competitive sugars, such as (GlcNAc)4, the monomer of (GlcNAc)4, and N-acetyllactosamine, can inhibit the binding of TL to rat intestinal rings, and reduce the binding values to 83%, 80%, and 75%, respectively. This confirms the targeted binding of TL to N-Glu- NAc. Unfortunately, TL suffers from cross-reactivity with mucus glycoprotein, leading to nonspecific binding. The investigation of lectin–sugar groups on the cell membrane has been the subject of relatively few studies compared to other types of mucoadhesive polymers, and has primarily been conducted using the intestinal, rather than buccal, epithelium.

Recently, lectin was used to estimate the ability of a polymer to mask the surface glycoconjugates and to determine the inhibition of surface-lectin binding of a biotinylated lectin from Canavalia ensiformis (sword bean). This represents one example of lectin used as a cell adhesion marker rather than a targeted delivery vehicle to the buccal cavity.

Nevertheless, lectin mediated bioadhesive polymers, as second-generation bioadhesives, contain an enormous potential for future uste in drug delivery which, unfortunately, have not yet been fully explored. The recent idea of developing lectinomimetics (lectin-like molecules) based on lectins, and even biotechnologically generated derivatives of such molecules, holds an interesting future for this class of bioadhesion molecules.

Computer-assisted molecular modeling has demonstrated that the lectin–sugar interactions contain only a small part of lectin which recognizes the sugar, while the remaining large portion of the glycoprotein is not involved in the detection and binding to the sugar. Therefore, the opportunity of designing lectinomimetics based on the active site of natural lectin seems very attractive, especially in view of its reduced toxicity/immunogenicity. This interaction would presumably create the same sugar recognition pattern that mediates cellular binding, and could potentially demonstrate wide applicability in the area of target-specific bioadhesive polymers. Possible application of lectin and lectin- like molecules to control targeting, binding, and cell internalization should be explored.

*Bacterial adhesion*

The adhesive properties of bacterial cells, as a more complicated adhesion system, have recently been investigated. The ability of bacteria to adhere to a specific target is rooted from particular cell-surface components or appendages, known as fimbriae that facilitate adhesion to other cells or inanimate surfaces. These are extracellular, long thread like pro-tein polymers of bacteria that play a major role in many diseases. Bacterial fimbriae adhere to the binding moiety of specific receptors. A significant correlation has been found between the presence of fimbriae on the surface of bacteria and their pathogenicity.

The attractiveness of this approach lies in the potential increase in the residence time of the drug on the mucus and its receptor-specific interaction, similar to those of the plant lectins. As an example, Escherichia coli have been reported to specifically adhere to the lymphoid follicle epithelium of the ileal Peyer's patch in rabbits. Additionally, different staphylococci possess the ability to adhere to the surface of mucus gel layers and not to the mucus-free surface. Thus, it appears that drug delivery based on bacterial adhesion could be an efficient method to improve the delivery of particular drugs or carrier systems. Bernkop-Schnu¨rch et al. covalently attached a fimbrial protein (antigen K99 from E. coli) to poly(acrylic acid) polymer and substantially improved the adhesion of the drug delivery system to the GI epithelium. In this study, the function of the fimbrial protein was tested using a haemagglutination assay, along with equine erythrocytes expressing the same K99-receptor structures as those of GI-epithelial cells. A 10-fold slower migration of the equine erythrocytes through the K99-poly(acrylic acid) gel, compared to the control gel without the fimbriae, was demonstrated, indicating the strong affinity of the K99-fimbriae to their receptor on the erythrocytes.

Some bacteria not only adhere to the epithelial cells, but also invade host cells using a mechanism resembling phagocytosis. Bioinvasive drug delivery systems have been developed based on this bacterial mechanism, where bacteria could be used as a vehicle to introduce drug compounds into host cells by means of multiple h1 chain integrin cell receptors, which are a member of the cell adhesion molecule (CAM) family. This idea has led to a patent by Isberg et al.

*Mucoadhesive polymers as enzyme inhibitors and permeation enhancers*

It has been shown that some mucoadhesive polymers can act as an enzyme inhibitor. The particular importance of this finding lies in delivering therapeutic compounds that are specifically prone to extensive enzymatic degradation, such as protein and polypeptide drugs. Investigations have demonstrated that polymers, such as poly(acrylic acid), operate through a competitive mechanism with proteolytic enzymes. This stems from their strong affinity to divalent cations (Ca2+, Zn2+). These cations are essential cofactors for the metalloproteinases, such as trypsin. Circular dichroism studies suggest that Ca2+ depletion, mediated by the presence of some mucoadhesive polymers, causes the secondary structure of trypsin to change, and initiates a further auto degradation of the enzyme.

The increased intestinal permeability of various drugs in the presence of numerous mucoadhesive polymers has also been attributed to their ability to open up the tight junctions by absorbing the water from the epithelial cells. The result of water absorption by a dry and swellable polymer is dehydration of the cells and their subsequent shrinking. This potentially results in an expansion of the spaces between the cells.

The use of multifunctional matrices, such as polyacrylates, cellulose derivatives, and chitosan, that display mucoadhesive properties, permeation-enhancing effects, enzyme-inhibiting properties, and/or a high buffer capacity have proven successful strategies in oral drug delivery. The inhibition of the major proteolytic enzymes by these polymers is remarkable and represents yet another possible approach for the delivery of therapeutic compounds, particularly protein and peptide drugs, through the buccal mucosa. Any newly developed excipients are likely to be subject to safety and toxicity testing to ensure the safety of these new-generation bioadhesive polymers. However, the level of testing depends on the compound.

Since lectins are found in many species in the plant kingdom (e.g. tomato, wheat germ, mistletoe), they are not likely to be toxic. The fact that the source plants can be consumed raw, e.g. tomato fruit, would seem to suggest the safety of lectins.

As mentioned previously, tomato lectin has been shown to bind to the surface of several cell monolayers, as well as rat intestinal epithelium without causing any harmful effects to the membranes. Another example is the clinical application of mistletoe lectin (Viscum album) for antitumor therapy in rabbits and cancer patients.

To achieve the desired level of cytoadhesion, genetically engineered lectins or lectinomimetics with reduced toxicity/immunogenicity could also be used. In contrast, haemagglutinin from red kidney beans (Phaseolus vulgaris) and bacterial adhesive proteins might require more extensive testing.

C. *Mucoadhesive dosage forms*

The primary objectives of mucoadhesive dosage forms are to provide intimate contact of the dosage form with the absorbing surface and to increase the residence time of the dosage form at the absorbing surface to prolong drug action. Due to mucodhesion, certain water-soluble polymers become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body for extended periods of time.

The mucosa lines a number of regions of the body including the gastrointestinal tract, the urogenital tract, the airways, the ear, nose, and eye. These represent potential sites for attachment of any mucoadhesive system and hence, the mucoadhesive drug delivery system may include the following[33]: Gastrointestinal delivery system, Nasal delivery system, Ocular delivery system, Buccal delivery system, Vaginal delivery System, Rectal delivery system. Selected commercial mucoadhesive drug delivery systems are summarized in table 2.

*Characterization of mucoadhesive dosage form*

No technology has still been developed specifically to analyze mucoadhesion. Most of the tests available were adapted from other pre-existing techniques but are useful and necessary for selecting the promising candidates as mucoadhesives as well as in elucidating their mechanisms of action. Various forces which can be characterizing adhesive properties are given in figure 5.

*Methods of analysis of mucoadhesion*

Since the early 1980s, a vast variety of methods to evaluate the potential mucoadhesive properties of new polymeric materials have been developed. The diversity in physical forms of the mucoadhesive devices invented led to the generation of a wide variety of techniques for mucoadhesion evaluation.

A large number of methods found in the literature are based on the measurement of the force necessary to separate a mucoadhesive material from a biological membrane. Peel, shear and tensile forces can be determined depending on the direction in which the mucoadhesive material is detached from the biological surface.

Peel forces are measured when evaluating mucoadhesive devices for buccal or transdermal applications. Within the shear strength tests, the Wilhelmy plate method developed by Smart et al. is one of the most remarkable methods. In this method, a glass plate coated with the mucoadhesive material to be tested is submerged in a mucin solution.

A microbalance connected to the plate measures the forces due to surface tension on the plate as the system containing the mucin solution is pulled away from the mucoadhesive material. This force measured is related to the wettability of the mucin on the polymer surface and corresponds to the adhesive force between the mucoadhesive polymer and the mucin glycoprotein.

Tensile tests have been widely used for the evaluation of a large diversity of mucoadhesive devices. For example, Ponchel et al. analyzed the tensile force required to separate a mucoadhesive tablet from animal mucosa. This force is then used to calculate the work of adhesion. This parameter has been extensively used as a good indicator of the mucoadhesive properties of a material and is calculated by the integration of the force vs. displacement curve obtained in the tensile experiments. Other notable mucoadhesion techniques include the method developed by Robinson et al., where human epithelial cells are labeled with fluorescent probes and placed in contact with a mucoadhesive polymer. The interaction between the epithelial cell membrane and the polymer is investigated. More recently, other methods used to examine the molecular interactions at cell surfaces include the force microscopy techniques.

Mikos and Peppas invented the flow channel method in which a mucoadhesive polymer particle is placed on a mucus surface in a Plexiglas_ channel. A laminar flow of air is directed over the microparticle, and photographs are taken to analyze the static and dynamic behavior of the polymer particle. Other techniques used for the evaluation of mucoadhesive particles include the electrobalance method and contact angle measurements. The falling film technique developed by Ho and Teng is also a remarkably simple method for the evaluation of mucoadhesive particles. In this method, spherical latex particles are coated with a mucoadhesive material and are suspended in a buffer solution of a known concentration. The particle solution is then pumped over a rat small intestine cut lengthwise and placed in a cylindrical channel. The eluted solution is collected and the remaining particles in the solution are counted. The portion of particles that remained adhered in the mucosal tissue is an indication of the mucoadhesive properties of the material tested.

Staining methods have also been developed for the evaluation of mucoadhesive polymers. A colloidal gold staining technique was developed by Park, where mucin-gold conjugates interacted with a hydrogel surface resulting in a red coloration. More recently, a direct-staining method to evaluate the attachment of a polymer to human buccal cells has been proposed. Hassan and Gallo reported the rheological method for mucoadhesion evaluation. This method is based on the idea that when a mucoadhesive polymer is mixed with mucin, there is a synergistic increase in viscosity. However, the contradictory results obtained in some experiments suggest that this method should not be used as a single technique to evaluate mucoadhesion.

Other techniques used to study the interaction between mucoadhesive polymers and mucin glycoproteins has been done by Huang et al. with the use of the surface force apparatus (SFA). The SFA measures the magnitude and distance dependence of the molecular force acting between two surfaces, with resolutions of the measured force up to 10 nN and distances up to 1 .Å.

Some in vivo methods to assess mucoadhesion properties of polymers include the gamma scintigraphy and the use of radioisotopes to measure the gastrointestinal transit of the mucoadhesive device.

A.*In vitro* and *ex vivo* tests

*In vitro/ex vivo* tests are important in the development of a controlled release bioadhesive system because they contribute to studies of permeation, release, compatibility, mechanical and physical stability, superficial interaction between formulation and mucous membrane and strength of the bioadhesive bond. These tests can simulate a number of administration routes including oral, buccal, periodontal, nasal, gastrointestinal, vaginal and rectal. The *in vitro* and *ex vivo* tests most prevalent in the literature are reported below.

*1. Techniques utilizing gut sac of rats*

The everted gut sac technique is an example of an *ex vivo* method. It has been used since 1954 to study in intestinal transport. It is easy to reproduce and can be performed in almost all laboratories.A segment of intestinal tissue is removed from the rat, everted, and one of its ends sutured and filled with saline. The sacs are introduced into tubes containing the system under analysis at known concentrations, stirred, incubated and then removed. The percent adhesion rate of the release system onto the sac is determined by subtracting the residual mass from the initial mass[34].

Other techniques use non-everted gut sac[35]. The sacs were sealed and incubated in saline. After a stipulated time, the number of liposomes adhered before ($N_0$) and after ($N_s$) incubation was assessed with a coulter counter and the percent mucoadhesive was expressed by equation (2).

$$\% \text{ adhesion} = \frac{N_0 - N_S}{N_0} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

The mucoadhesive effect of a system can also be evaluated by increases in gastrointestinal transit. Fluorescent tracers are incorporated into a system and quantified them by fluorescence spectroscopy in the stomach and intestinal mucus as a function of time.

*2. Tests measuring mucoadhesive strength*

Most *in-vitro/ex-vivo* methodologies found in the literature are based on the evaluation of mucoadhesive strength, that is, the force required to break the binding between the model membrane and the mucoadhesive.

Depending on the direction in which the mucoadhesive is separated from the substrate, is it possible to obtain the detachment, shear, and rupture tensile strengths as indicated in Figure 5[36].The force most frequently evaluated in such tests is rupture tensile strength. Generally, the equipment used is a texture analyzer or a universal testing machine. In this test, the force required to remove the formulation from a model membrane is measured, which can be a disc composed of mucin, a piece of animal
mucous membrane, generally porcine nasal mucus or intestinal mucus from rats. Based on results, a force-distance curve can be plotted which yields the force required to detach the mucin disc from the surface with the formulation, the tensile work (area under the curve during the detachment process), the peak force and the deformation to failure. This method is more frequently used to analyze solid systems like microspheres, although there are also studies on semi-solid materials (mini-matrices) [36-39].

In addition to rupture tensile strength, the texture analyzer can also, as inferred by its name, evaluate the texture of the formulations and assess other mechanical properties of the system. A mobile arm containing an analytical probe forces down into a sample held in a flask placed on the equipment's platform. Speed rate, time and depth are preset.

From the resulting force-time and force-distance plots, it is possible to calculate the hardness (force required to reach a given deformation), compressibility (work required to deform the product during the compression), and adhesiveness (work required to overcome the attraction forces between the surfaces of sample and probe). Using this technique, it is possible to perform a previous evaluation of the material's adhesive capacity, evidencing mucoadhesion properties[38-41].

3. *Imaging methods by AFM, CFSLM, MPEM*

Optical microscopes offer insufficient resolution for studying effects at a molecular level. For such investigations, a resolution at micro or nanometric level is needed. Electronic microscopy gives a larger view, but the environmental conditions in which the sample must be submitted are far from the physiological conditions. For instance, the samples are analyzed in a vacuum chamber and generally are covered with a metallic film to avoid changes caused by the electronic rays. SEM in the studies of mucoadhesive gives the topology information in vitro, but the nature of the dosage form in vivo and the nature of transport across the biological barriers are missing.

Atomic force microscopy (AFM) is a relatively new technique that overcomes such restrictions, because it can be used under any environmental conditions, in air, liquids or vacuum. It enlarges more than $10^9$-fold, which enables visualization of isolated atoms and offers a tridimensional image of the surface. The equipment has a support combined with a probe perpendicularly attached to it. This tip moves toward a plane parallel to the sample, acquiring its topographic characteristics and the tip position is recorded by an optic deflection system: a laser beam is reflected onto the support and its position is then further reflected by a mirror reaching a photodiode sensor. A force-distance curve is plotted to measure the forces between this tip and the surface of interest. This curve is then used in bioadhesion studies. This entails, coating the tip in adhesive material which is generally spherical in shape and then the interaction with the surface, in this case the mucous membrane, can be measured.

Besides AFM, there are other techniques using photographic images, such as fluorescence microscopy and confocal laser scanning microscopy (CSLM)[42-43]. B. R. Masters have described about confocal microspcopy right from its history to the present updates[44]. Hirofumi Takeuchi et al has used confocal imaging to see the nature of mucoadhesive microspheres ex-vivo in the gut of rats[45]. Here a tungsten or lazer illuminates and detects the scattered or fluorescent light respectively within the vesicles.

A set of conjugated apertures, one for illumination and one for detection of light function as spatial filters.

In confocal microscopy, lateral and axial resolutions are enhanced when compared to standard light microscopy. The axial resolution is responsible for identifying the lodging position of the vesicles ever deep within the tissues.The main advantage of confocal microscope is its ability to optically section thick specimens. Real time video frame can be captured with a low light video camera which inturn can be connected to a video recorder. Video frames give a live demonstration of the pathway and nature of the transportation of vesicles. Fluorescent dyes for detection used are Calcien AM (for green fluorescent), Rhodamine – 123, Rhodamine – DHPE, fluoroscien – DHPE, nile red[46].

Multi-Photon excitation microscopy (MPEM)is another tool which can be conveniently used to study the living tissues. So the microspheres inside the tissues also can be studied. The important work of Denk, Strickler and Webb which was published in Science in 1990 launched a new revolution in nonlinear optical microscopy. They implemented multi-photon excitation processes into microscopy by integrating a laser scanning microscope and a mode-locked laser which generates pulses of near-infrared light. The pulses of red or near-infrared light (700 nm) were less than100 fsec in duration and the laser repetition rate was about 80 MHz.

These pulses have sufficiently high peak power to achieve two-photon excitation at reasonable rates at an average power less than 25 mW that induces minimal photo damage to many types of Biological samples. However, highly pigmented cells and tissues could be subjected to photo-induced thermal damage. The potential benefits of two-photon excitation microscopy include reduced photo bleaching of the fluorophores, improved background discrimination and minimal the photo damage to living cell specimens[47-51].

*3. Falling Liquid Film Method*

The chosen mucous membrane is placed in a stainless steel cylindrical tube, which has been longitudinally cut. This support is placed inclined in a cylindrical cell with a temperature controlled at 37 ºC. An isotonic solution is pumped through the mucous membrane and collected in a beaker (Figure 6). Subsequently, in the case of particulate systems, the amount remaining on the mucous membrane can be counted with the aid of a coulter counter. For semi-solid systems, the non adhered mucoadhesive can be quantified by high performance liquid chromatography. In this later case, porcine stomach, intestinal and buccal mucus were tested, and also jejunum from rabbits. The validation of this method showed that the type of mucus used does not influence the results. The release systems tested were precursors of liquid crystals constituted by monoglycerides. This methodology allows the visualization of formation of liquid-crystalline mesophase on the mucous membrane after the flowing of the fluids and through analysis by means of polarized light microscopy[52].

## Conclusion

Mucoadhesive drug delivery system shows promising future in enhancing the bioavailability and specific needs by utilizing the physiochemical characters of both the dosage form and the mucosal lining. It has to be noted that only a moist surface can bring the mucoadhesive nature of the dosage form.

Mechanism of mucoadhesion is backed up by ionic bond, covalent bond, Vander Waal bond and hydrogen bond. Ionic and covalent bonds results in very strong mucoadhesive property. Mucoadhesion commence with wetting which is described as contact stage.

In the consolidation stage lot of physiochemical interaction takes place. While considering a formulation development of mucoadhesive drug delivery dosage form, several physiological factors also has to be considered at the site of action. Several synthetic and natural polymers are considered to have complying properties of mucoadhesion. While performing gastro retentive mucoadhesive *in-vivo* tests, it should be proved that the dosage form is no more available in the stomach after the desired period.

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Table 1: Composition of mucosal epithelia

| *Contents* | *Optimum concentration (%)* |
|---|---|
| Water | 95% |
| Glycoprotiens and lipids | 0.5-5% |
| Mineral salts | 1% |
| Free proteins | 0.5-1% |

Table 2: Commercial Mucoadhesive Drug Delivery System

| *Drug* | *Mucoadhesive Polymers* | *Application Site* | *Name and form* |
|---|---|---|---|
| Triamcinolone acetonide | Hydroxypropyl cellulose, cabopol 934 | Oral cavity | Attach tablet |
| Nitroglycerin | Synchron (modified HPMC) | Buccal | Susadrintablet |
| Prochlorperazine Maleate | Ceronia, Xanthum Gum | Buccal | Buccastem tablet |
| Beclomethasone dipropionate | Hydroxypropyl cellulose | Oral cavity | Salcoat powder spray |
| | Sodium CMC, pectin, and gelatin in poly-ethylene mineralail base | Oral cavity | Orabase gel |
| | Sodium CMC.pectin, and gelatin in polyisobutylene spread ontopolyethylene film | Oral cavity | Orahesive bandage |
| Beclomethasone dipropionate | Hydroxypropyl cellulose | Oral cavity | Rhinocort powder |
| | Polyacrylic acid | Vaginal | Replens gel |
| Aluminium hydroxide | Sucrose octasulfate | Gastrointestional ulcers | Sucralfate |

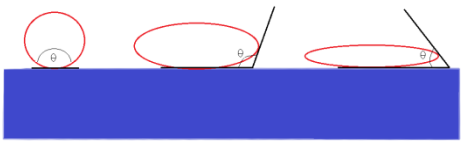

Figure 1: Influence of contact angle between dosage form and mucous membrane

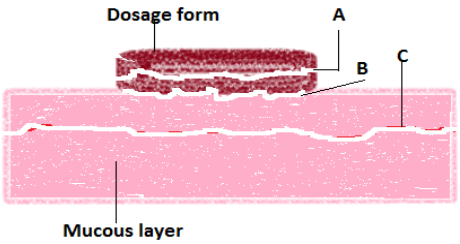


Figure 2: Progressions of bond rupture at various regions: fracture within hydrated layer of mucoadhesive dosage form (A); fracture at interface between dosage form and mucous layer (B); fracture within mucous layer (C).

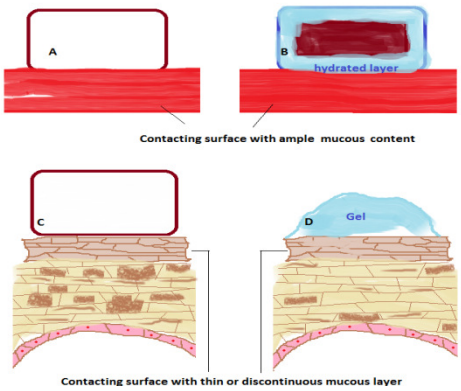


Figure 3: Various approaches of mucoadhesion- A,B represent considerable mucus layer surface and C,D represent thin or discontinuous mucus surface : clockwise from top left- dosage form in dry/semi hydrated state(A); fully hydrated (B); dry /partially hydrated (C); fully hydrated (D)

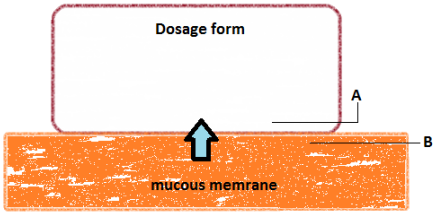


Figure 4: Dehydration theory of mucoadhesion explaining demonstrating water movement from the mucous region to mucoadhesive dosage form.

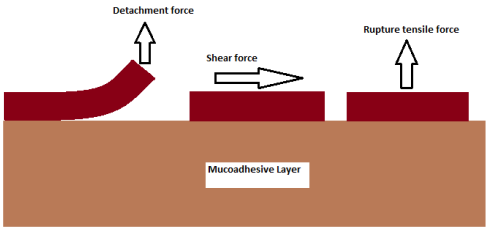


Figure 5: Various forces characterized in adhesive strength which is the basic for mucoadhesive tests.

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