DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(1&2), 143-194 (1993)

MUCOADHESIVE DRUG DELIVERY SYSTEMS

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ABSTRACT

Pharmaceutical aspects of mucoadhesion have been the subject of great interest during recent years because mucoadhesion could be a solution for bioavailability problems that result from a too short length of stay of the pharmaceutical dosage form at the absorption site within the gastro-intestinal tract.

This paper describes some aspects of bioadhesion such as mucus structure, stages of adhesion and the theories proposed that attempt to explain the adhesion mechanism. The factors that affect the bioadhesive power of a polymer, the methods that permit the evaluation of a bioadhesive

system and the methods for surface characterization of biomaterials are discussed. Finally, the various polymers used and the bioadhesive systems designed for several therapeutic purposes are presented.

INTRODUCTION

A few years ago interest in the adhesion of dosage forms to epithelial surfaces was aroused by the possibility of deliberate contact between oral dosage forms and the gut wall for the purpose of retarding the rate of transit through the gastro-intestinal tract[1-4], and also by the possibility of moistened dosage forms accidentally adhering to the esophagus or other epithelial surfaces. Adhesive preparations for topical treatment of stomatitis[5] and the adhesive nature of transdermal patches are important, as is the adhesion of film coating to tablet surfaces. Adhesion of erythrocytes and bacterial cells[6-7] to polymer surfaces is of increasing importance in the understanding of blood compatibility, polymers and bacterial infection mediated by catheters.

CONCEPTS

Adhesion can be defined as the bond produced by contact between a pressure-sensitive adhesive and a surface[8]. By another definition it is the ability of an adhesive to stick to another surface[9]. It can also denote a state in which two surfaces are held together[10].

Many years ago ASTM[11] extended the concept of adhesion, defining it as the state in which two surfaces are held together by interfacial forces, which may consist of valence forces, interlocking action or both.

Good[12] defined bioadhesion as the state in which two materials, at least one of which being of a biological nature, are held together for an extended period of time by interfacial forces. By another definition, bioadhesion[13-14] is the ability of a material (synthetic or biological) to adhere to a biological tissue for an extended period of time. These definitions include a large number of adhesion phenomena[14]; like, adhesion of cells to one another, or the adhesion of various shellfish to rocks. Different types of adhesion have different medical implications. For instance, minimal adhesive strength is desired as critical to preventing unwanted thrombus formation in cardiovascular devices, plaque buildup on dental prostheses, and bacterial fouling of heat exchanges[15], while maximum adhesion and immobility are desirable for orthopedic and dental implants.

In biological systems, four types of bioadhesion could be distinguished[16]: a) adhesion of a normal cell on another normal cell, b) adhesion of a cell with a foreign substance, c) adhesion of a normal cell to a pathological cell, d) adhesion of an adhesive to a biological substrate.

For drug delivery purposes, the term bioadhesion implies attachment of a drug carrier system to a specific biological location. The

biological surface can be epithelial tissue, or it can be the mucous coat on the surface of a tissue. If adhesive attachment is to a mucous coat, the phenomena is referred to as mucoadhesion. Bioadhesion can be modeled after a bacterial attachment to tissue surfaces, and mucoadhesion can be modeled after the adherence of mucus on epithelial tissue[17].

INTEREST OF MUCOADHESIVE SYSTEMS

Mucoadhesion could resolve several problems of controlled reels systems[18]: a) it localizes drugs in a particular region, thereby improving and enhancing bioavailability for those drugs with bioavailability problems; b) the strong interaction between a polymer and the mucus lining of a tissue helps increase contact time and permit localization, an essential factor when modification of tissue permeability is important for delivery; c) it inhibit the metabolizing of enzymes in a localized area; and d) it frees agents locally for the purpose of modulating antigenicity.

Furthermore, in many sustained-release products a relatively fast-acting formulation produces undesirably high plasma concentrations, while a slow-acting formulation fails to reproduce the bioavailability of the multiple dosage regimen. This is because the conventional approach to sustained-release formulation is generally unsuitable for certain classes of drugs or active ingredients which are not adequately absorbed during circulation through the organism. This can be due to either

physico-chemical properties of the drugs or to their requirement for a particular site of absorption[19].

For instance, in the cases of:

1) Buccal administration, which is of great interest because it provides the possibility of avoiding either destruction by gastrointestinal liquids or hepatic first-pass inactivation. However, it is often difficult to maintain the tablet in a suitable place in the mouth or even to fail to swallow it.

Anders, et al.[20] obtained a short exposure time of buccal tissues to thyrotropin and prolactin, when they used a disc-shaped, water-soluble paper filter and then impressed this on the buccal tissue. A similar situation was obtained by Pimlott and Addy[21] with isosorbide dinitrate, using a primitive drug delivery system. In contrast, Nagai and Machida[22], showed that mucoadhesion can be used as a platform for local delivery of drugs to the mouth with concomitant improvement in therapy or, alternatively, a decrease in body load of the drug.

2) In Rectal administration, for systemic activity purposes, might be important to maintain the pharmaceutical dosage form in the lower part of the rectum, where the hemorrhoidal veins escape from the hepatic pass.

3) In Vaginal administration, where it is highly desirable that the dosage form not be eliminated prematurely from its activity site.

4) Nasal administration. In recent years the possibility that the intranasal administration route might be useful for many compounds which are not

absorbed orally has received a great deal of attention[23-24]. Studies often conclude that a nasal delivery system could be an extremely useful route for systemic administration. But, Hassain et al[25], for instance, in nasal absorption studies of sustained-release dosage forms of propanolol in rate, closed the nasopalature with an adhesive agent to prevent drainage of the drug form.

Recently, Gonda and Glipps[26] developed a mathematical model to describe the rate processes involved in the behavior of drugs in a delivery system placed into the human nasal cavity. The effect of bioadhesive carriers was successfully simulated by reducing the mucociliary clearance rate constants for the transport from the posterior part of the nose into the gastro-intestinal tract. The simulation shows that bioadhesion improves bioavailability and reduces the variability in absorption which might be caused by a variable pattern of deposition in the nose.

5) Ocular administration. A necessary condition for the activity of the dosage form is the guarantee of keeping it in place for a sufficient length of time.

Topical delivery of drugs to eye is significantly constrained by tear turnover[27-28], instilled solution drainage[29], and absorption at other than target times. Of these, drug loss through instilled solution drainage and tear turnover, (i.e. clearance from the front of the eye), are the most important[30]. Retaining the drug on the front of the eye through the use of a mucoadhesive, which attaches

to the conjunctival mucus, would substantially improve ocular drugs in terms of their bioavailability.

6) Oral administration. Emptying of the stomach and intestinal peristaltism can, unfortunately, displace the active ingredient from its resorption site expelling the conventional controlled-release system before drug release occurs. Therefore, certain medicinals dissolve better in the acidic medium of the stomach rather than in the neutral alkaline environment of the intestine. Obviously, the passage of a controlled-release dosage form into the neutral or alkaline region of the G.I. tract could result in a potential decrease in the dissolution and absorption rates of the sustained-release agent[31].

Furthermore, many active ingredients are principally absorbed from the upper portion of the small intestine, and it is not possible to establish a uniform plasma level by the administration of a conventional-release system which may deliver the active ingredient beyond the site of absorption.

Experiments in rats[32] showed unequivocally that mucoadhesive polymers were retained in the stomach for an extended period of time and that stomach emptying of mucoadhesive-treated particles was prolonged to more than a day. Furthermore, administering a poorly bioavailable drug, chlorotiazide[33], with a mucoadhesive dosage form led to a substantial improvement in bioavailability for these drugs.

In light of these considerations, it is important to know both the mechanism(s) of adhesion

as well as ways to utilize mucoadhesion as a platform for both local and systemic delivery of drugs[13,34].

MUCUS LAYER

In most instances the mucoadhesive polymer is in contact with a soft tissue. Thus, the tissue layer responsible for formation of the adhesive interface is mucus. The term mucus usually refers to the layer covering the mucosa.

The composition of mucus varies widely depending on animal species, anatomical location, and whether the organism is in a normal or pathological state[35-41]. It is secreted by the goblet cells lining the epithelia or by special exocrine glands with mucus cells acini.

The lubrication properties of mucus secretions are a result of their viscous and gel-forming properties[43] and general stickiness[44]. An example of this is the sticking of gastro-intestinal mucus glycoproteins to the surface of cells in tissue culture[45].

Recently a great number of articles[18,44,46-48] have extensively described the characteristics and composition of mucus. Therefore we will only refer to the most important aspects.

Mucus glycoproteins are high-molecular proteins possessing attached oligosaccharide units. These units contain an average of about 8-10 monosaccharide residues of five different types. They are as follows, with the systematic name given in parentheses where different: L-fucose (6-deoxy-L-

galactose); D-galactose; N-acetyl-D-glucosamine (2-acetamide-2-deoxy-D-glucose); N-acetyl-D-galactosamine (2-acetamide-2-deoxy-d-galactose); and sialic acid. In humans the only important sialic acid is N-acetylneuramic acid (5-acetamide-3,5-dideoxy-D-glycero-D-galacto-nonulosonic acid), although in animals a number of other sialic acids occur, including N-glycollyneuramic acid and various O-substituted deviates[41,49]. Amino acids are principally serine, threonine and proline.

Sialic acid has an axial carboxyl group and it is an important source of negative charge for many mucus glycoproteins. Fucose possesses an equatorial methyl group which confers a degree of hydrophobicity on that region of the molecule. The acetamide groups of N-acetylhexosamines play a similar role. Galactose is an important constituent of mucus glycoproteins, and it is important for this monosaccharide to possess both axial and equatorial hydroxyl groups[41].

Many of the terminal residues in the oligosaccharide side chains are negatively charged sialic acids[14].

Linkages between the protein cores are of the o-glucidic type, between N-acetylgalactosamine and serine or threonine[44,50-51]. The entangled nature of mucus is due to disulfide linkages (intrachain). Macromolecular associations are due to physical entanglement stabilized by electrostatic interactions or the other non-covalent constants between the oligosaccharide chains or between chains and the protein cores of the molecule[18,47].

The principal differences between the mucus glycoproteins of this study are molecular weight[47,52], length and number of chains and distance between chains[44].

The physicochemical properties of mucus are almost certainly dependent upon both the protein and carbohydrate components of mucus glycoproteins. Proteolytic enzymes[40,53-54] or the cleavage of disulphide bonds destroy the physicochemical properties of the mucus. On the other hand, the mucus glycoprotein is largely composed of carbohydrate, and it is the carbohydrate that is in immediate contact with the environment. Consequently, the chemistry of the glycoproteins is, to a considerable extent, the chemistry of the oligosaccharide units.

This mucus layer, which covers the epithelial surface, has various roles: a) Protective: resulting particularly from its hydrophobicity, and protecting the mucosa from the lumen diffusion of hydrochloric acid from the lumen to the epithelial surface[47]; b) Barrier: the role of the mucus layer as a diffusional barrier in tissue absorption of drugs and other substrates is well known[44,55-58], as it influences the bioavailability of drugs[44,59-64]. To this effect, it has also been proven that bile salts can modify the permeability of physiologic membranes, including gastric mucus, by liquefying its structures[65-66]; c) Adhesion: mucus has strong cohesional properties and firmly binds to the epithelial cell surface as a continuous gel layer. One must consider the structure and density of oligosaccharide side chains of the cell surface, their interaction with lipids and

proteins, and their "fuzzy coat" glycocalyx in developing mechanisms of bioadhesion[18].

MUCOADHESION

Logically, for bioadhesion to occur, a succession of phenomena , whose role depends on the nature of the bioadhesive, is required. The first stage involves an intimate contact between a bioadhesive and a membrane, either from a good wetting of the bioadhesive surface, or from the swelling of the bioadhesive. In the second stage, after contact is established, penetration of the bioadhesive into the crevice of the tissue surface or interpenetration of the chains of the bioadhesive with those of the mucus takes place,. Low chemical bonds can then settle.

One of the most important factors for bioadhesion is tissue surface roughness, because many time solid surfaces are not actually planar. The innumerable small hills, valleys, and crevices in the surface create problems which must not be neglected if strong, durable, adhesive joints are desired. A viscous liquid can appear to spread well over a solid surface and yet have many gas pockets or voids in small surface pores and crevices where the liquid adhesive has formed a mantle over neighboring peaks. Without an adhesive that spreads spontaneously over the solid, there is no certainty that intimate contact of liquid and solid interface will occur.

Griffith[67] showed that adhesive joints may fail at relatively low applied stresses if cracks, air

bubbles, voids,inclusions, or other surface defects are present.

A rough surface may be defined in terms of the ratio of maximum depth (d) to maximum width on surface roughness[68]. If this ratio is less than 1:20, insignificant roughness for adhesive purposes is present; in this case, viscosity and wetting power are the most important factors for satisfactory bioadhesion.

Many theories have been proposed to attempt to explain the adhesion mechanism. Initially, adhesion is possible between materials without specific chemical affinity, but a good wetting and sufficient spreading are necessary to guarantee molecular contact between the two phases.

A. Wetting: We distinguish:

A.1. For a liquid bioadhesive: Young[67] provided the first good approach for describing the wetability and spreadability of a liquid on a solid, by discussing the contact angle of a liquid and the equilibrium of a drop resting on a flat solid surface under the action of three surface tensions: a) surface tensions at the interface of the liquid and the vapor phases (γ_L); b) surface tensions at the interface of the solid and vapor phases (γ_s); c) and surface tensions at the interface of the solid and the liquid phases (γ_{SL}). At equilibrium[69]:

$$\gamma_S = \gamma_{SL} + \gamma_L \cos \theta$$

complete wetting is signified if the contact angle between a liquid and a solid is 0_o; if this angle approaches 180°, insignificant wetting is signified.

The type of wetting in which a liquid spreads over the surface of a solid is referred to as spreading wetting. The tendency for spreading may be quantified in terms of the spreading coefficient[70]:

$$S = \gamma_L \ (\text{Cos } \theta - 1)$$

If the contact angle is larger than 0°, the term (Cos θ - 1) will be negative, as will the value of S. The condition for complete, spontaneous wetting is therefore, a zero value for the contact angle.

A.2. For a solid bioadhesive: The work of adhesion can be expressed in terms of surface and interfacial tension. The work of adhesion, which is the energy required to break the attraction between unlike molecules, is given by[67,71]:

$$W_A = \gamma_M + \gamma_B - \gamma_{MB}$$

where subscripts M and B refer to the biological membrane and the bioadhesive formulation, respectively. On the other hand, the work of cohesion or work required to separate like molecules, is given by:

$$W_L = 2\ \gamma_M \quad \text{or} \quad W_C = 2\ \gamma_B$$

The term (W_A - W_C) is known as the spreading coefficient (S), and for a bioadhesive material spreading on a biological substrate, is given by:

$$S_{B/M} = W_A - W_C = \gamma_M + \gamma_B - \gamma_{MB} - 2\ \gamma_B = \gamma_M - (\gamma_{MB} + \gamma_B)$$

One sees that $S_{B/M}$ should be positive for a bioadhesive material to adhere to a biological membrane.

Spreading may also be thought of in terms of surface-free energy. In this case, $S_{B/M}$ should be negative. In other words, the surface-free energy of the new

system is reduced when a bioadhesive material adheres to a biological membrane[72].

Also, critical surface tension was related to surface cell spreading. The range for the best overall effectiveness as indicated by the critical surface tensions at which cell spreading begins is between 20-30 dynes/cm[16,73-74]; although Jendressen and Glantz[75] saw a bioadhesive range of 32-50 dynes/cm for the clinical adhesiveness of tooth structure.

Recently Mikos and Peppas[76] measured the surface tensions of various aqueous mucin solutions prepared with crude mucin from the stomach portion (PSM) or mucin from bovine submaxillary glands (BSM) and different concentrations of NaCl, at constant or varying pH, using the pendent drop method. They concluded that surface tension was independent of added electrolyte concentration pH and of mucin concentration; it depended only on the source of mucin. The value of surface tension was smaller for BSM.

Wachem et al.[77] studied in vitro interaction of human endothelial cells with polymeric substances possessing different wetabilities in a culture medium containing serum. They found that moderately wettable polymers showed optimal adhesion, and that spreading and proliferation to cells and adhesion decreased or disappeared with either very hydrophilic or very hydrophobic polymers. In a homologous series of cellulosic polymers the authors observed an increase in bioadhesive strength as the contact angle increased.

On the other hand, for a solid material, the role of water in the bioadhesion mechanism is of primal importance, as shown by Chen and Cyr[78]. The authors observed that maximum wet adhesive strength is attained when perfect matching of active adhesive sites is achieved in the presence of an optimum amount of water at or near the interface. If insufficient water is used to hydrate the dry hydrocolloid, active wet adhesion sites are not completely liberated and exposed for interaction. An excessive amount of water, on the other hand, causes over-extension of the hydrogen bonds and other adhesive forces leading to a weakening of the adhesive.

Dittgen et al.[79] observed the influence of the concentration of aqueous solutions of mucus excipient on bioadhesion in vivo. Maximum bioadhesion corresponded to a concentration of the excipient from 0.15% to 23.7%, and to a viscosity of the aqueous solutions from 0.1 Pa.S to 3.6 10^6 Pa.S..

Smart et al[80] observed no significant difference in the adhesive forces obtained when different mucosa-adhesive materials underwent hydratation in contact with, or before contact with " homogenized " mucus samples. They also showed variation on adhesiveness when they used mucus samples and various gel samples, due, among other things, to variations in water availability in the medium. Similarly, Leung and Robinson[81], in studies of a series of cross-linked copolymers with acrylic acid-methyl methacrylate, showed that the polymer-

mucine tensile strength increase with degree of hydratation.

This situation is very important, for instance, in the case of intraoral bandages, since the diffusion of saliva into the bandage can cause significant changes, including an eventual loss of adhesiveness.

A second theory that attempts to explain adhesion was proposed by Voyutski[82] and Bueche et al[83]. According to their theory, polymer chains and mucus commingle to a sufficient degree to create a semi-permanent adhesive bond.

Later, Prager and Tirrell[84] extended the theory of diffusion-interpenetration to a simpler version of the " reptation " model proposed by Gennes[85], saying that when a bioadhesive and glycoproteinic network are brought into contact, the polymer chains penetrate the mucus at rates which essentially depend on the diffusion coefficient and time of contact. This diffusion coefficient, in turn, depends on the value of molecular weight between crosslinks, and decreases significantly as crosslinking density increases[54,64,86-87].

The electronic theory of adhesion was suggested by Derjaguin and Surilga[88]. According to this theory, electron transfer occurs upon contact of an adhesive polymer with a mucus glycoprotein network because of differences in their electronic structures. This results in the formation of an electrical double layer at the interface. Adhesion occurs due to attractive forces across the double layer.

Kemball and Huntsberger[89-90] described the adsorption theory. According to this theory, after an initial contact between two surfaces the material will adhere because of surface forces acting between the atoms in the two surfaces. Two different types of chemical bonds can be distinguished[14,68]: a) Primary chemical bonds of covalent nature, undesirable in bioadhesion because their high strength results in permanent bonds[17]; b) secondary chemical bonds having many different forces of attraction, including electrostatic forces, Van der Waals forces and hydrogen and hydrophobic bonds.

A theory for adsorption of a polypeptide chain capable of undergoing the coil-ß-structure transition on a solid planar surface has been developed by Birshtein et al.[91].

Recently, Ponchel et al[92] showed that bioadhesion results from a compromise between the chemical interaction theory (theory of absorption for the interface between functional polymer groups and mucus), and the theory of polymer chain interpenetration in mucus.

Finally, the fracture theory is related to the separation of two surfaces after adhesion. Fracture strength, equivalent to adhesive strength, is given by [14,47]:

$$G = \sqrt{E \epsilon / L}$$

where E is Young's modules of elasticity.

ϵ is the fracture energy, and

L is the critical crack length when two surfaces are separated.

As mentioned by Ahagon and Agent[93-94] the fracture energy of an elastomer network (T_o) is given by:

$$T_o = K M_{c\ 1/2}$$

where K is a constant dependent on the density of the polymer, the mass, length and effective flexibility of the monomer unit, and bond dissociation energy; and M_c is the average molecular weight of chains between crosslinking points. T_o of an elastomeric network increases with M_c of the network stands[93-94].

Typically, the force of attachment of an adhesive polymer to mucin is sufficiently strong so that removal occurs primarily through mucin turnover.

During in vitro adhesion tests, Ch'ng et al[32] observed that when polycarbophyl was detached from tissue , mucus remained bound to the polymer and the break occurred within the mucus network. Thus, it can be deduced that the interaction force between this polymer and mucus was greater than the mucus-mucus cohesive force in a rabbit's stomach.

Many factors can affect the bioadhesive power of a polymer; some are dependent on surrounding media and others on the nature of the polymer.

Robinson and his group[32] observed a significant effect of pH in studies of polyacrilic polymers crosslinked with -COOH groups because of the influence of pH on the surface change of both mucus and polymer[32,92]. Mucus has a different charge density, depending on pH, because of differences in dissociation of functional groups on the carbohydrate moiety and in the amino acids of a polypeptide backbone.

Also, this group observed[32] that the pH of the medium was critical for the degree of hydratation of highly crosslinked polyacrylic acid polymers, increasing between pH 4 and pH 5, continuing to increase slightly at pH 6 and pH 7; and decreasing at more alkaline pH levels. Also, they showed that the force required to separate the polycarbophyl from freshly excised rabbit stomach tissue was maximal at pH 5 and 6, and minimal at pH 7.

James et al.[95] investigated the effect of pH on the rheology of purified and unpurified gastric hog mucin and found that the intrinsic viscosity was not affected by the changes of pH in unpurified mucin; however, purified mucin showed a decrease in viscosity at low pH. Forstner et al[96] observed a decrease in specific viscosity of semipurified gastric hog mucin as pH increased from 4 to 9, although the solubility of the mucin was not strongly affected by changes in pH. They also found that the mucin was not susceptible to changes in shear rate at pH 7, which they attributed to an instability of the structure of mucin or to a greater sensitivity to shear at physiological pH values.

Finally, Smart et al[80] showed that a low pH favors adhesion between gelatin gels prepared at various pH's and plates coated with P75 SC MC and tragacanth. Adhesion was maximum at pH's below the separation points of the gelatin molecules, since at these pH's gelatin carries a positive net charge while SCMC and tragacanth carry a negative net charge.

As mentioned by Gurny et al[97], it seems that adhesive strength increases as the molecular weight

of an adhesive polymer increases to 100.000 and beyond this level there is not much effect. Although a critical length of the molecules is necessary to produce the interpenetrating layer and molecular entanglements between the bioadhesive and the substrate, one must also consider the size and configuration of the interpenetrating adhesive macromolecules. Chen and Cyr[78], for example, point out the case of PEG polymers: Carbowax 20M, with a molecular weight of 20.000, has no wet adhesive property. At a molecular weight of 200.000 its adhesiveness is improved, and at 4.000.000 it is an excellent adhesive.

Smart et al[80] found that for sodium carboxy methyl cellulose, optimum adhesive forces were obtained with a molecular weight >/ 78.600 daltons.

Besides molecular weight and chain length, spatial information about the molecule is also important.

For instance, Chen and Cyr[78] point out that dextrans of molecular weights as high as 19.500.000 have similar adhesive strengths to that of PEG with a molecular weight of 200.000. There, due to the helical conformation of dextrans, many of the adhesively active groups are " shielded " inside the coils and so do not actively participate in the process primarily responsible for adhesion, unlike PEG polymers which have a linear conformation.

Bremecker[98] reports that there is an optimum concentration of polymer that corresponds to the best bioadhesion. In high concentration systems, the adhesive strength drops significantly because the

chains available for interpenetration are not numerous. It has been pointed out also that excessive crosslinking of the polymer adhesive does not contribute to bioadhesion for the same reasons[99]. Gurny et al[97] indicate this, but this result seems to be of importance only for relatively liquid bioadhesive forms; still, Duchêne et al[47] show that for solid dosage forms such as tablets, the higher the polymer concentrations, the stronger the bioadhesion.

Adhesion properties vary according to the degree of hydratation. Chen and Cyr[78] indicate that adhesion is maximum at a certain degree of hydration. When the degree of hydration is high, adhesiveness is lost probably due to the formation of slippery, nonadhesive mucilage in an environment of a large quantity of water or near the interface. For example, researchers[97] have shown in studies with hydrocolloids (more specifically Orobase^{r}), that although the wet adhesive strength (measured as stress at break), which developed as the hydrocolloid components absorbed water, increased with increasing degree of swelling, excessive water content led to an abrupt drop in adhesive strength. This is clearly an indication of disentanglement at the hydrocolloid/tissue interface due to low concentrations of the active components, (if we accept the diffusion theory, according to which bioadhesion is a result of interpenetration of polymer chains throughout the bioadhesive interface of substrate).

If the diffusion theory is accepted, molecular flexibility is another parameter which should be considered in the process of adhesion[18].

Smart et al[80] studied the influence of a gel network on adhesiveness using a gelatin gel and gelatin sol. Although it appeared that the presence of a gel network was not a firm requisite, the adhesive forces were considerably lower on the sol than on the gel.

METHODS FOR MEASUREMENT OF MUCOADHESION

Various methods for studying bioadhesion have been described and can be classified in two large groups: a) in vitro methods, most of which require the use of an artificial biological medium such as mucus[80] or saliva[100-101]; and b) in vivo methods.

Most in vitro methods are based on the measurement of either shear or tension stress[78,81,10]. For instance, Reich et al[102] designed and constructed a device for measuring the force of adhesion between plastic material and endothelium after contact, using a metal or glass fiber deflection technique for measuring force.

Smart et al[80,103] developed a method for the measurement of bioadhesiveness which is a modification of the Wilhelmy method for the measurement of superficial tension. In this method the plates are coated with the polymer to be tested and immersed in a temperature-controlled mucus solution. In this method, the force required to detach the glass plate coated with the test material was measured. A similar method was used by Ishida et

al[104] to measure the adhesiveness of oral mucus ointments.

Gurny et al[97] used a tensile tester (Instron, Model 1114) equipped with a custom-made cell for measuring adhesive strength.

Additional in vitro bioadhesive tests have been described[105], most of which are peeling tests based on peel force tests run at short contact times and low contact pressures before the bond is completely formed[106].

Wang and Llewellyn-Thomas[107] have developed a technique which utilizes the dorsal skin of Wistar rats. A thin layer of bioadhesive material is placed on the skin, and the pressure required to disrupt an incision closed by this particular adhesive admixture is recorded from the reading registered on the gauge of the tensiometer in pounds per square inch.

Robinson and his group[34] developed a fluorescence probe technique using cell cultures which indirectly measures the binding between a polymer and epithelial cells. The binding of a polymer to a lipid bilayer of a cell membrane containing the fluorescent probe pyrene, which compresses the lipid bilayer, results in a change in florescence. The change in florescence is proportional to the degree of binding of the polymer to the cell membrane. This can be explained by the fact that photo-excited pyrene can react with non-excited monomer to form a complex called excimer. It is also possible to obtain information on cell polarity from the peak ratio measurement of monomer fluorescence, since the pyrene monomer is characterized by three well-defined peaks. Thus, the

peak intensity ratio of II/I can be used as a measure of polarity of the probe environment, designated as the Py value.

Recently a similar procedure has been proposed by Park[108] called "mucin-gold" staining and is used for the quantitative comparison of mucoadhesive properties of various hydrogels. The technique employs red colloidal gold particles which are stabilized by the adsorbed mucin molecules (mucin-gold conjugates). Upon interaction with mucin-gold conjugates , mucoadhesive hydrogels develop a red color on the surface. Park points out the following advantages over using animal tissues: 1) The colloidal gold staining technique is simple to perform. No special instrument other than a spectrophotometer is necessary for the technique; 2) The experimental cost is much lower, and the cost of colloidal gold staining is negligible; 3) This technique allows the study of interaction between mucin molecules and polymer chains at the molecular level to take place; 4) Experimental conditions can be maintained and the results are highly reproducible; 5) It is possible to make mucin-gold conjugate in sufficiently large quantities so that the mucoadhesive properties of a large number of different polymers can be compared at the same time under the same conditions.

The Robinson group[109] used a modified surface tensiometer to measure the force required to separate a polymer from freshly excised rabbit stomach tissue. Similar techniques had been used by Ishida et al[110] using mouse peritoneal membrane, and by Ponchel et al[92,111] using ox sublingual mucosa.

Forget et al[112] proposed a measurement system for assessing the adhesivity of mucoadhesive tablets containing polyacrylic acid which uses a stainless steel sieve as the adhesive surface, .

As an alternative to exploring bioadhesion Baszkin and Lyman[113] proposed investigating the surface energetics of polymer surfaces, studying the adsorption/desorption of proteins.

Marvola et al[114] developed two systems for measuring the adhesion of a dosage form (non-bioadhesive systems) to the esophagus, using segments of pig esophagus maintained at 37° C in oxygenated tyrode solution. Swisher et al[115] as well as Al-Dujaili et al[116] employ analogous methods for the same purpose.

Proust et al[117] used a device to study the adsorption of bovine submaxillary mucin from sigma on mica surfaces to obtain information on the tear film rupture process.

A method[118] has been proposed to simulate the real behavior of a gastro-intestinal bioadhesive system on mucus. The apparatus consists of a thin channel filled with a mucus gel or natural mucus solution. The channel is thermostated and equipped with a transparent cover which can be removed by a handle. The system is connected through a valve to a fluid source which may be a gas or a visco elastic liquid. The channel is placed on an optical microscope. A spherical polymer particle of known weight is placed on the surface of the mucin, and the lid is closed. The distance traveled by the particle is measured, as well as the time for detachment and the type of motion.

In the same sense a novel in situ method has been proposed recently by Rao and Buri[119]. In this technique, the glass spheres or drug crystals were first coated with the polymers to be tested. Later, known amounts of these coated particles were placed on rat jejunum or stomach and placed in a humid environment. The tissue was then washed with phosphate buffer (for jejunum) or diluted HCl (for stomach) at a constant rate. The percentage of particles retained on the tissue was regarded as an index of bioadhesion.

Robinson and his group[32] developed an in vivo method using male Sprague Dawley rats. A capsule containing solid control or test material was surgically inserted into the stomach of anesthesized rats. The rats were permitted to awaken and at suitable times the animals were sacrificed, and the stomach and small intestine were removed. The intestine was cut into 20 equal segments and the radioactivity was measured in each segment of the stomach.

Davis[120] described a noninvasive technique for examining the bioadhesive characteristics of polymers using gamma scintigraphy.

METHODS TO SURFACE CHARACTERIZATION OF BIOMATERIALS

In recent years the surface characterization of biomaterials has been strongly emphasized. Three primary reasons why surface characterization is important to biomaterials science are[121] : 1) surface identification (chemistry, structure, and

reproducibility assurance); 2) contamination detection (reproducibility assurance); and 3) correlation between surface structure and bioavailability.

To this effect, Ratner[121-122] has been accumulating various techniques for surface characterization of biomaterials, classified in the following groups: a) Thermodynamic analysis; b) Surface electrical properties; c) surface chemistry analysis; d) Spatially resolved surface chemistry analysis; e) Surface topography; and f) Surface crystallinity and atomic organization. Ratner proposes[122] Electron Spectroscopy for Chemical Analysis (ESCA) as the most valuable single method available for characterizing the surfaces of biomaterials, whereas Miller and Peppas[123] propose X-ray Photoelectron Spectroscopy. The latter may be used to : 1) Study the surface chemistry of the bioadhesive polymer; 2) Establish possible interfacial bonding between polymer and artificial mucus for in vitro studies; and 3) identify the site of failure of the bioadhesive bond.

BIOADHESIVE POLYMERS

Polymers which can adhere to either hard or soft tissue have been used for many years in surgery and dentistry[124-125]. Among these " super glues ", polymers and monomeric alpha-cyanoacrylate esters have been most frequently investigated[126-127] and used[128].

Other synthetic polymers such as polyurethanes, epoxy resins, polystyrene, acrylates, and natural-products cement were also extensively investigated, as were glues[129-134]. Recently, an examination of polymers that adhere to the mucin-epithelial surface of the G.I. tract was begun.

A bioadhesive which can be useful in oral drug delivery by prolonging GI transit time and improving oral drug absorption should ideally be nontoxic, nonabsorbable from the GI tract, preferably form a strong noncovalent bond with mucin-epithelial cell surfaces, adhere quickly to moist tissue, allow easy incorporation of drug and offer no hindrance to its release, possess specific sites of attachment, and be economical[16,32,135].

Robinson and his group[34], using the fluorescence technique, concluded that:

1. cationic and anionic polymers bind more effectively than neutral polymers.

2. polyanions are better than polycations in terms of binding/potential toxicity, and further, that water-insoluble polymers give greater flexibility in dosage form design compared to rapidly or slowly dissolving water-soluble polymers.

3. anionic polymers with sulphate groups bind more effectively than those with carboxilic groups.

4. degree of binding is proportional to the charge density on the polymer.

5. highly binding polymers include carboxymethyl cellulose, gelatin, hyaluronic acid, carbopol, polycarbophyl.

For the purpose of acquiring a better understanding of the relationship between polymer structure and bioadhesive potential, this group also synthesized a series of anion cross-linked swellable polymers of the polycarbophil family[32], and measured their ex vivo bioadhesion. They showed that poly (acrylic acid/divinylbenzene), polycarbophil and poly (acrylic acid-2,5-dimethyl-1,5-hexadiene) have bioadhesive characteristics, whereas poly (2-hydroxyethylacrylate) or poly (HEMA) do not. Amberlite 200 and gelatin showed poor or non-existent bioadhesive qualities. It is necessary to point out that the role of pH on bioadhesion is of great importance, with maximum adhesion being observed from pH 5 to 6.

When they compared the possibilities for bioadhesion during gastro-intestinal transit between polycarbophyl, poly-(methacrylic acid/divinyl benzene) and amberlite, it was polycarphophil which had the best bioadhesive qualities in the stomach as well as in the small intestines.

Using their method, Rao and Buri[119] showed that policarbophil and sodium carboxymethylcellulose adhered more strongly to mucus than to hydroxypropylmethylcellulose, methylcellulose or pectin. Better adhesion occurred in the stomach than in the intestine.

Polycarbophil[33,136] is a synthetic polymer composed of polyacrilic acid loosely cross-linked with 0.5-1% (w/w) divinyl glycol (3,4-dihydroxy-1,5-hexadiene). It consists of particles that swell but are insoluble in water. The particles are also

insoluble, but may swell to varying degrees in common organic solvents, strong mineral acids, and bases. Swelling characteristics in water depend on the pH and the ionic strength of the test solution, with swelling increasing as pH increases. At low pH (pH 1-3), polycarbophil absorbs ≈ 15-35 mL of water per gram of resin, whereas in neutral or basic media it can absorb ≈ 100 mL per gram. This compound is approved for use in humans in antidiarrheal and laxative products since, concerns about the toxicity of the polymer are minimal.

The following is an outline of the mechanisms of attachment of polycarboxilic acids to mucin[17]: the polymer undergoes swelling in water and this permits entanglement of the polymer chains with mucus on the surface of the tissue. The unionized carboxylic acid groups bond to the mucin molecule by means of hydrogen bridges.

Other studies have been undertaken to classify bioadhesive polymers[78,80]. They demonstrate the important bioadhesive power of carboxymethyl cellulose and carbopol 934. Many researchers[92,110,137-139] used a mixture of carbopol 934 and hydroxypropyl cellulose, but carbopol was the bioadhesive agent and the cellulosic derivative was the hydrophilic matrix.

Recently Smart and Kellaway[140] showed that an adsorbed film of the mucosa-adhesive polymer carbopol 934P resulted in almost complete retention of the resin particles (amberlite ion exchange resin IRA 400) within the stomach of male mice at the end of the 1 hour experimental time. Korsmeyer and Peppas[141] employed a copolymer poly (therma-co-NVP) and Gurny

et al[97] used a polythyelene gel containing sodium carboxymethyl cellulose.

Other systematic studies of bioadhesion have been performed by Marvola et al[142] but for different purposes, since they are concerned with assessing the role of formulation factors on oesophagal drug bioadhesion and they deal more especially with the effects of film coating agents.

MUCOADHESIVE DOSAGE FORMS

Mucoadhesive dosage forms can be regarded as a new type of preparation that may make treatment more effective and safe not only for local diseases, but also for systemic diseases.

a. Oral administration: A primary objective of using mucoadhesive formulations orally would be to achieve a substantial increase in length of stay of the drug in the gastro-intestinal tract.

In 1985, Longer et al[33] showed that albumin beads containing chlorthiazide, when mixed with equal sized particles of polycarbophil at a ratio of 3:7 (w/w) (albumin beads:polycarbophil), and administered orally in the form of capsules to rats, that in vitro release studies, the albumin beads and bioadhesive dosage form offered sustained-release for ≤ 8h.. However, more than 60% was released after 2h, indicating that the release rate was still quite rapid and also that the presence of polycarbophil did not affect the rate of release of drug from the beads. In vivo studies showed that nearly 90% of the beads in the polycarbophil-albumin bead dosage form

remained in the rat stomach. In the absence of polymer, the majority of beads moved at least half-way down the small intestine, with some moving farther. Also, they observed that the technique of using a bioadhesive in drug delivery significantly improves therapy by increasing the duration of action and bioavailability over that which is attained with a typical sustained-release dosage form.

When these experiments[17] were repeated in dogs, less satisfactory results were obtained. The explanation for the difference in findings stems from the difference in the amount of soluble mucin in the stomach of the rat versus that of the dog.

The influence of the putative bioadhesive polycarbophil on the gastric emptying of a pellet formulation had been investigated by Khosla and Davis in 1987[143]. The gastric emptying of pellets, labelled with a gamma emitting radionuclide, was measured in human subjects, using the technique of gamma scintigraphy. Similar rates of emptying for polycarbophil formulation and control formulation indicated that their admixture with polycarbophil did not retard the gastric emptying of pellets in fasted subjects. On the other hand, Russell and Bass[144] reported that 50% of a 90g polycarbophil meal emptied within 4h in canine gastric acid. Again the explanation for the difference in findings stems from the amounts of polycarbophil used in these studies.

Very recently Ito et al[145] developed magnetic granules containing ultrafine ferrite , brilliant blue FCF and bioadhesive polymers (10:1:9 w/w) surmising a possible application for targeting

therapy for esophageal cancer. When 5 mg of granules containing a mixture of HPC and Carbopol 934 (6:4 w/w) was flushed into an agar-gel tube with 20 mL of 0.65% HPC solution, about 90% of the granules were held in the region of the applied magnetic field. When the granules were administered to rabbits with about 2 mL of 0.65% HPC solution via catheter and without anesthesia, nearly all of the granules were held in the region 2 hours after administration with magnetic guidance for the initial 2 minutes.

b. Buccal administration: The mouth is an available, but not extensively utilized area of the body for drug delivery. Some emergency drugs are routinely administered orally, but it is generally not considered a useful area for drug delivery. During the last year, because of the higher permeability of mouth tissue in comparison to skin, high vascularity bypass of first-pass metabolism and accessibility, considerable attention is being focused on this area for drug delivery purposes.

The first oral adhesives used in the mouth were developed in dental practice. One example is the orahesive bandage[146] composed of gelatin, sodium carboxymethylcellulose and polyisobutylene backed by a layer of polyethylene film on one side and a layer of removable-release paper on the other.

Nagai's group has been in the forefront of development of bioadhesive controlled-release systems. In 1981[110] they attempted to develop a new oral mucosal dosage form with a view to solving the problems of the administration of insulin by injections. This new form consisted of a core-base,

which contained cacao butter, insulin and additive, and a peripheral-base, which contained a mixture of hydroxypropyl cellulose-H (HPC) and carbopol-934 (CP) in a ratio 1:2 HPC and CP.This mixture was slowly compressed on a hydraulic press. Unfortunately, the percentage of insulin absorbed in this dosage form was about 0.5% compared with the amount absorbed through intramuscular injection of insulin.

One year later[138] they attempted to develop a dosage form containing a local anesthetic for toothaches, using lidocaine as a model drug in HPC, CP as a peripheral base, and directly compressing these with a hydraulic press. Finally, a third layer was applied, consisting of a freeze-dried mixture of HPC and CP, added to magnesium stearate (1:1). This dosage form could afford a long-acting local anesthetic action, especially if lidocaine can be advantageously replaced by dibucaine in order to obtain a better anesthesia.

For the treatment of aphthae, Nagai[104], using carbopol 934 as the muco-adhesive, showed that the release of prednisolone from an ointment-type oral mucosal dosage form containing 30% carbopol was better than the original base.

Schor et al.[147] developed a nitroglycerin bioadhesive tablet, using a range of polymers made from naturally occurring materials (Synchron^{r}) which can be mixed directly with an active pharmaceutical substance and directly compressed into tablets for the treatment of angina pectoris. The buccal tablet was quite small so that it would adhere to the buccal mucosa and not require adhesives to hold it in place.

The tablet completely dissolved over a period of hours to produce a steady, high level of clinical activity over a period of 5 to 6 hours.

Triamcinolone acetionide has been formulated[22] using the principles of mucoadhesion for the treatment of aphthous stomatitis. The dosage form is a double layered tablet of small dimensions. The upper layer is colored and consists of lactose and has no adhesive properties. Its role is to permit drug diffusion out of its activity site and to allow an easy placing of the bioadhesive tablet. The lower layer contains the active ingredient and the mucoadhesive polymer hydroxypropyl cellulose and carbopol 934. It is commercially available in Japan under the name of AFTACH^{r} .

Also, Nagai et al[148] describes two examples of "semi-topical" drug delivery systems: (a) a mucosal adhesive dosage form of lidocaine for toothaches using HPC and CP, and (b) an adhesive gingival plaster containing prostaglandin F2 for the facilitation of tooth movement in orthodontic treatment.

Yotsoyanagai et al[149] designed a mucoadhesive using moderately water soluble polymer films containing analgesics and antibiotics for pain relief and which aids in the healing of lesions. The film consisted of hydroxypropyl cellulose containing tetracaine, thiamphenical and triacetin.

Robinson et al[17], in conjunction with scientists at 3M/Riker laboratories, developed a buccal patch using polycarbophil. In dogs, the patch remained in place for approximately 17 h., regardless

of food or drink, and similar findings were observed in humans.

More recently, Deasy and O'Neill[150] developed a bioadhesive dosage form for peroral administration of Timolol base. The core containing 10mg methylene blue and 10mg Precirol was lightly compressed on a 4mm fat-faced punch and die set. The core was centered on the 8.5 mm lower punch in a die and overfilled with 120mg of bioadhesive polymer (HPC 80mg and carbopol 934, 40mg) which was then lightly compressed. The cap layer of 20mg magnesium stearate was added, and the composite device was compressed using a medium concave upper punch under 106Kg cm^{-2} . Results in humans showed that an average of 34% of the drug loading was absorbed in an apparently zero order manner over 3h. This was less than in dog studies and was presumably due to the poorer permeability of the human gingiva compared to the oral mucosa of dog. The addition of sodium laurylsulphate 0.1% to the core enhanced penetration, increasing the mean quantity absorbed over 3h to 61% of the drug loading.

Saton et al[151] studied factors affecting the bioadhesive properties of compressed tablets consisting of hydroxypropyl cellulose and carboxyvinyl polymer. The interpolymer complex formation between HPC and CP is particularly noteworthy and was confirmed by turbidity and viscosity measurement. Maximum turbidity was found at the weight ratio of HPC-CP 3:2 in the acidic medium (pH 3.0). No solid complex formation was observed in the higher pH region (pH 4.5 and 6.0). When the weight fraction of HPC in samples was from 10% to

60%, the viscosity of the supernatant in the HPC-CP solution was observed to be almost the same as that of the medium. When the FT-IR spectra of HPC-CP solid complexes were determined, the peak at 1710 cm^{-1} nearly disappeared at the ratio of 3:2 (HPC-CP), suggesting that a stable solid complex was formed at this weight ratio. Although the weakest adhesion force was observed at a mixing ratio of 3:2, the researchers note that this method for measuring adhesion might be very important.

Finally, Collins and Deasy[152] developed two and three layered devices by filing the desired proportions of the components (cromadol, carbopol, cetylpyridinium chloride, flavorings, HPC, magnesium stearate, precirol, spermaceti was and talc) of each layer into a punch and die set. After each layer was added, the fill was tapped down and lightly compressed. Finally, the completed compact was compressed at a force 212 Kg cm^{-2} in an infrared press. In vitro dissolution studies on two-layered devices showed that when the content of the matrix in the upper layer with HPC was increased from 40% to 60%, drug release was reduced. When talc was introduced into the upper layer in three-layered devices the release profile was very similar to that of two-layered devices. Little effect on release rate was obtained when natural spermaceti was substituted by synthetic spermaceti or Precirol. Finally, when the quantity of drug in the upper layer of the device was increased from 2.5 mg to 5mg, no significant difference in the release profile was obtained. The device offered considerable improvement over the

proprietary product in sustaining salivary levels of drug in the therapeutic range, however only ~52% of the drug loading was released in vivo over a 3 h. period compared to ~90% in vitro. The workers concluded that the production process could be simplified by manufacturing only the drug containing layer by compression.

c. Sublingual administration: Using the principle of bioadhesion Gurney et al[97] attempted to deliver febuverine sublingually. The bioadhesive polymer system was prepared from a polyethylene gel containing various amounts of sodium carboxymethylcellulose as the adhesive, and hydrolysed gelatin as the water-sensitive material to ensure rapid swelling. They found that the relative adhesive bond strength of the various formulations was dependent on the concentration of NaCMC, showing a maximum at about 20 wt%. Also, optimal drug release rate was achieved in formulations with NaCMC concentration in the range of 12-15 wt%.

d. Nasal administration: In recent years, intranasal administration, which might be useful for many compounds which are not absorbed orally, has received a great deal of attention. Nagai et al[153] in their study of dogs with powder dosage forms of insulin, using a freeze-dried powder with carbopo 934, obtained the same blood concentration of insulin as with an intravenous injection of three times higher dosage.

Morimoto et al[154] developed a bioadhesive system for nasal administration of nifedipine, using a mixture of drug (10 mg/mL), PEG 400 and carbopol 941

(50:50), and obtaining a relatively high and sustained drug plasma concentration .

In two articles, Illum et al[155-156] demonstrated the bioadhesive properties of severe microsphere (Albumin, starch and DEAE-dextran microspheres) for nasal use. The half life of clearance for starch microspheres was found to be in the order of 240 min., compared to 15 min. for the liquid and powder control formulations.

e. Ocular administration: Hui and Robinson[157] showed, using progesterone as the model drug,that the area under the curve of an aqueous humor drug concentration versus time plot was 4.2 times grater than conventional suspensions in rabbits.

f. Cervix administration: Machida et al[137] developed a topical, disk-like dosage form for carcinoma coli. The 300 mg flat-faced disks measured 13 mm in diameter and about 2.0 mm in thickness and were made by direct compression of a mixture of bleomycin hydrochloride and a combination of HPC and other water-soluble polymers. A combination of HPC and CP934 was chosen as the vehicle, and the amount of BLM released from the preparation increased remarkably with an increase in concentration of HPC. In contrast, the water-absorbing property increased with an increase of CP.

More recently, Le Joyeux et al[158] developed a bioadhesive tablet of metronidazole for oral or vaginal administration, containing 50% drug, 37.5% HPC and 12.5% carbopol 934P. The tablets were 12mm in diameter and 2 mm thick. It seems that the presence of a large quantity of mucus at the interface

protects the bioadhesive system from the effects of the surrounding medium.

g. Rectal administration: Leede et al[159] proposed cylindrical hydrogels using hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) as crosslinking agents including antipyrine and theophylline as model drugs, for rectal administration.

In conclusion, the advantages of bioadhesive dosage forms make further study in this field extremely important. We truly hope that in the near future, these new dosage forms will be a reality for use in oral administration and become an alternative to controlled-release dosage forms.

ACKNOWLEDGMENTS

Dr. Jiménez-Castellanos appreciates the research grant received from D.G.I.C.T. of the Spanish Ministry of Education and Science.

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