POLYMER STRUCTURAL FEATURES CONTRIBUTING TO MUCOADHESION

by

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To our Father in Heaven

POLYMER STRUCTURAL FEATURES CONTRIBUTING TO MUCOADHESION

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Under the supervision of Professor Joseph R. Robinson

Mucoadhesive polymers and/or copolymers can be used as platforms for controlled drug delivery. In order to use mucoadhesives to prepare dosage forms, a better understanding of the structure-property relationship between mucoadhesives and mucin-epithelial layer is required.

The influences of charge and charge-related properties to mucoadhesion were studied by synthesizing a series of 0.2% cross-linked copolymers (acrylic acid-methyl methacrylate).

The quantitative characterization of the synthesized cross-linked copolymer networks was successfully done by using infrared spectrophotometry. The experimental percent compositions of acrylic acid and methyl methacrylate in different cross-linked copolymers were found to be in good agreement with starting fractions of the component parts.

The viability of rabbit stomach tissue used in mucoadhesion experiments was done by using the two-dye method and the bioluminescence method. The rabbit stomach tissue was found to be viable during the course of the experiment in chilled and aerated normal saline solution.

There are two kinds of adhesive strengths, tensile and shear,

involved in mucoadhesion. Both shear and tensile strengths were found to increase with applied weight with an limiting plateau value. The applied weight might influence the mucoadhesive strength by affecting the first stage of mucoadhesion, which is the establishment of intimate contact between the mucoadhesive and mucin. In the second stage of mucoadhesion, secondary bonds formation, the interpenetration process might increase contacting area between mucoadhesive and mucin and enhance formation of secondary bonds.

Tensile strength was found to be a time dependent and temperature dependent process and was found to be proportional to the mean diffusional path. It is possible that the expanded nature of both mucin and polymer networks influence the process of interpenetration/interdiffusion.

The average mesh size of the copolymer network was found to be proportional to the percent composition of the acrylic acid in the network. A relationship between tensile stress and average mesh size was proposed. It seems that mucoadhesive with desired mucoadhesive strength can be designed by controlling the percent of charge groups and corresponding mesh size of the network.

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ABBREVIATIONS:

x-AA/MM	:	cross-linked acrylic acid-methyl methacrylate copolymer
x-AA	:	cross-linked polyacrylic acid
x-MM	:	cross-linked polymethyl methacrylate

I. INTRODUCTION

In the last decade, bioadhesive polymers/copolymers have received considerable attention as platforms for controlled drug delivery.[5-9] The reason for the interest is that bioadhesive polymers and/or copolymers fulfill the following desirable features of a controlled release system: 1) localization in specified regions to improve and enhance bioavailability of drugs; 2) optimum contact with the absorbing surface to permit modification of tissue permeability as might be required for absorption of peptides/proteins and ionized species, or to inhibit protease activity; and 3) prolonged residence time to permit once daily dosing for the eye, mouth, gastrointestinal tract, vagina, etc., such that patient compliance can be improved. When applied to a mucosal epithelium, a bioadhesive synthetic hydrogel adheres primarily to the mucus layer, and this phenomenon is referred to as "mucoadhesion".

Adhesion is an interfacial phenomenon influenced by surface energies. When an adhesive bond is established between two materials, the total surface energy of the system is diminished, destroying two free surfaces and creating a new interface.

When adhesion involves biological surfaces, the phenomenon can be termed bioadhesion. A bioadhesive substance has the ability to interact with biological materials and is retained on the biological substrate for an extended period of time.

Perhaps one difference between bioadhesion and adhesion is that the former usually occurs in the presence of water.[1] Bioadhesives may exhibit poor adhesive characteristics when they are dry, and the terms 'wet adhesion'[2], 'hydrocolloid' and 'hydrogel'[3] have often been used.

A large number of vegetable gums and animal proteins were explored as adhesive pastes when moistened or hydrated with water. The first registered products of this sort were Orahesive powder® and Orabase®, the latter consisting of very finely ground pectins, gelatins and sodium carboxymethylcellulose (NaCMC) in a polyethylene/ mineral oil gel base.[4]

II. BACKGROUD

A. Mucus Layer

A continuous layer of mucus covers all the internal tracts of the body and is the first layer that interacts with foreign materials, e.g., food, drugs, bacterial organisms, and chemicals etc. Mucin glycoproteins have strong attachment properties not only to each other but also to other bioadhesive molecules and firmly bind to the epithelial cell surface presenting as a continuous, unstirred gel layer over the mucosa. Thus, a thorough understanding of the mucus layer is important.

1. Secretion (Production)

Mucus in higher organisms is usually defined as

the viscous fluid lining the epithelium of the gastro-intestinal, respiratory and genito-urinary tracts. Mucus is a mixture of large glycoproteins (mucins), water, electrolytes, sloughed epithelial cells, enzymes, bacteria and bacterial products, and various other materials, depending on the source and location of the mucus.[10] In the form in which mucus is generally secreted, the glycoprotein contitutes less than 5% of the total weight of mucus.[11] Most of the fluid is physiological saline, with some diffusible macromolecules.[12]

Mucins are synthesized either by goblet cells lining the mucus epithelium or by special exocrine glands with mucus cell acini.[10] Mucus-secreting cells are located in various organs of the body, e.g., salivary gland, stomach, small intestine, large intestine, gall-bladder, larynx, trachea, bronchus, uterine cervix, vagina and ovary.[13] These secretory cells are not uniformly distributed.[14] It was found that carbonoxolone and prostanglandins E_1 and E_2 can cause an increase in the rate of synthesis and secretion of gastric mucus.[15]

Mucin-containing vesicles bud off the Golgi apparatus and accumulate in secretory granules. The maximum size of the mucus glycoprotein intracellularly is determined by the size of the granules (200-1800nm)[16] and the concentration of the mucus glycoprotein inside the granule.[12] These granules then migrate towards the apex of the cell and discharge mucin out of the cell by exocytosis.[13,14,17,18] In secretion, the glycoprotein is closely

associated with proteins and separation is difficult even though no permanent bond exists.[19,20]

After exocytosis, the secretion forms discrete spherical boluses with diameters ranging from 0.1 to 25μm.[14] The increment of the radii of the boluses follows first-order kinetics of radial expansion. The corresponding volume expansion is approximately 1:600.[14] There is remarkable resemblance between the swelling of exocytosed mucins and that of artificial polymer gels, suggesting that the volumetric expansion is due to swelling and not to extrusion of cytoplasmic material.[14]

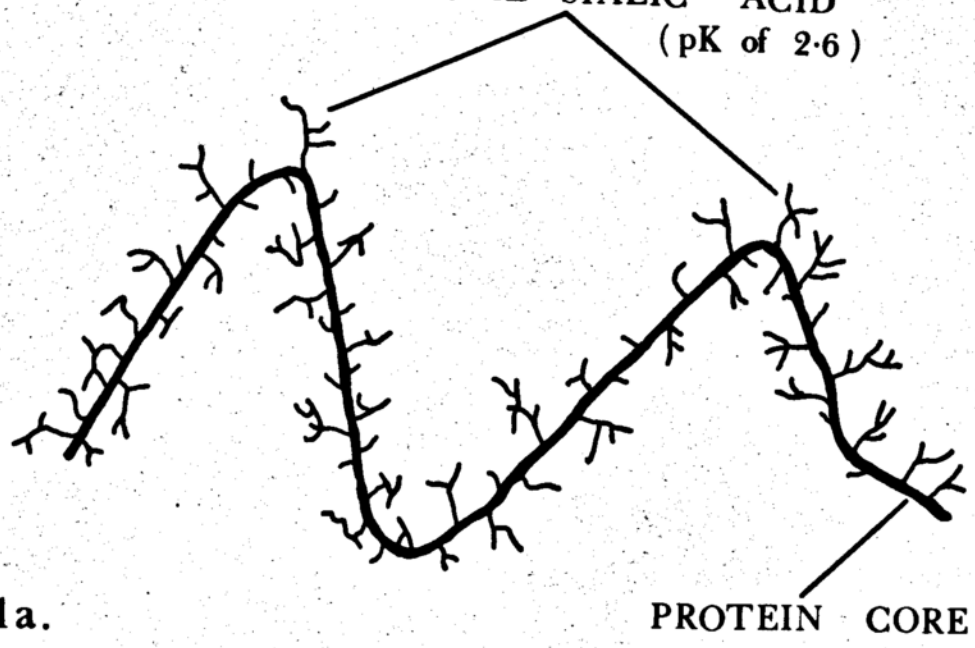
2. Mucus Composition

a. Structure of Mucin Glycoprotein

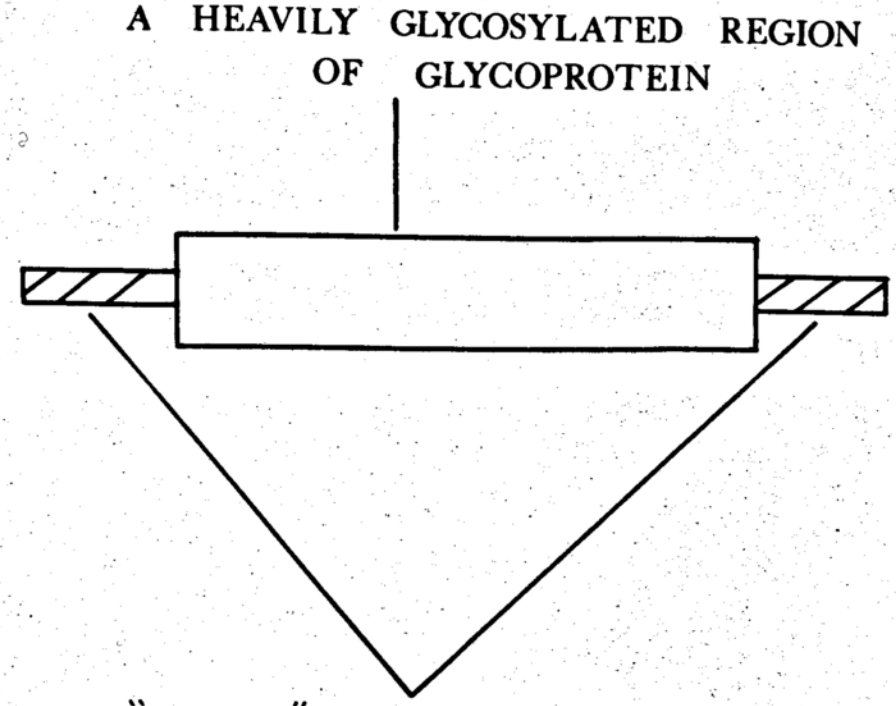
The basic component of all mucus is the mucin glycoprotein. There are about 160-200 oligosaccharide side-chains in the glycosylated region of the glycoprotein,[11,12,21] constituting 50-80% of its weight.[22,23] On the average, each oligosaccharide unit consists of eight to ten monosaccharide residues (M.W. = 320 to 4500[24]). These oligosaccharides are covalently linked to hydroxyamino acids, serine and threonine, along the polypeptide backbone,[25-27] as shown in figure 1a,[28] with sialic acids[29,30] (figure 2a) or L-fucose[21,31,32] (figure 2b) located at the terminal ends of the oligosaccharide chains. Sulfate esters are a component of some

Figure 1a: Schematic structure of mucin
(From reference 28)

Figure 1b: The mucus glycoprotein basic unit
(From reference 21, 22 and 35)


OLIGOSACCHARIDE SIDE CHAINS WITH TERMINAL SIALIC ACID
(pK of 2·6)
PROTEIN CORE

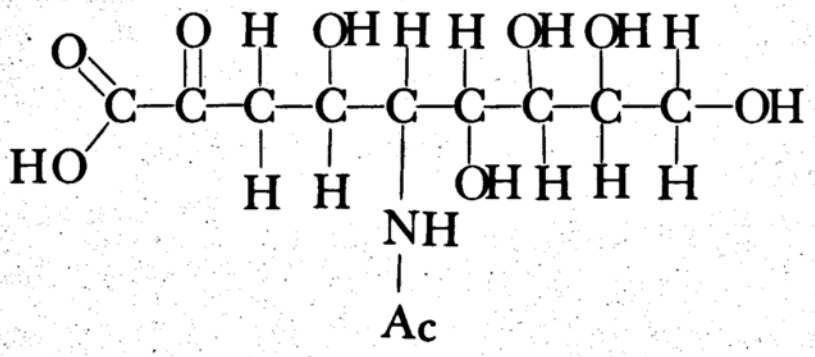
1a.


A HEAVILY GLYCOSYLATED REGION OF GLYCOPROTEIN
"NAKED" PROTEIN REGIONS

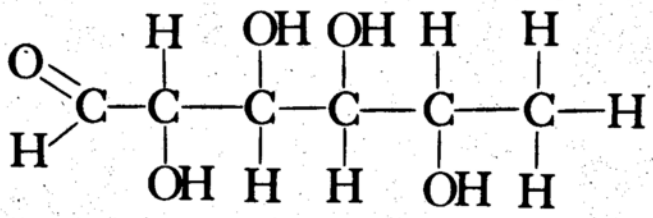
1b.

Figure 2a: Structure of sialic acid (keto form)
(acylated neuraminic acid)

Figure 2b: Structure of L-fucose



2a.



2b.

mucin molecules and are linked to either galactose or N-acetylglucosamine.[60] These carbohydrate side-chains form a dense, fairly uniform hydrophilic cover for different glycoproteins, although considerable microheterogeneity in length and complexity exists among the carbohydrate side chains.[33,34] The tightly packed oligosaccharides protect the protein core from digestion by proteases.

Mucins are polydisperse with respect to molecular sizes and oligosaccharide sequences and chain lengths.[10] The essential features of the glycoprotein unit providing a common link between all mucus secretions are shown in figure 1b.[21,22,35] A sialic acid : fucose ratio of 0.98 to 1.75 was reported by Wolf et al.,[36] thus 50 to 60% of the terminal groups are sialic acid. The 'naked' protein regions at the ends of the chain are not covered by a carbohydrate side chain and are thus susceptible to proteolytic digestion.[12,37-39] The sugar bearing section was shown to contain practically no cysteine residues while almost all of the cysteine's are found in the 'naked' peptide regions,[21] and the 'naked' regions are very high in charged amino acids, particularly aspartic acid.[12] Thus, the 'naked' peptide regions are generally considered to be involved in cross-linking via disulfide bridges between the mucin molecules.[12,39] It has generally been described that there is one 'naked' peptide region (bottle-brush model)[37,38] or two 'naked' regions, one of which is much longer than the other (rolling-pin model).[12]

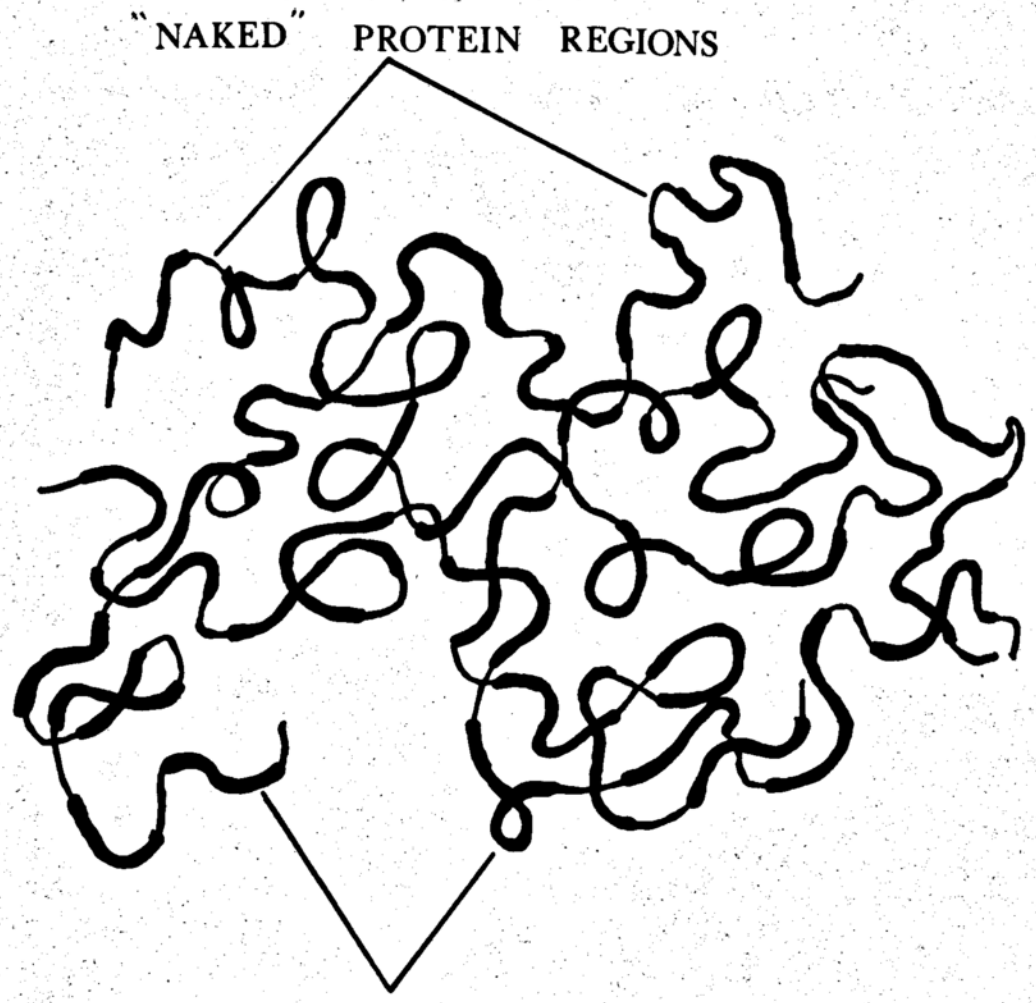
Specific non-covalent association between carbohydrate side chains and peptide backbone is geometrically feasible.[40] A highly extended and flexible molecular conformation is also suggested for mucus glycoproteins.[40]

b. Structure of Mucus Network

Under the electron microscope, mucus appears to consist of two elements: small dots and fine filaments.[41] A sol and gel form of the mucoid samples were further proposed by Bhaskar et al.[42] There are two forms of the glycoprotein; one of these is of density 1.511g/ml, stable at high pressure, and has a more compact structure, and the other is of density 1.485 g/ml.[43] The mucus is a viscoelastic gel.[12] Such a system can be viewed as the macromolecules all linked together via cross-linking to give rise to an infinite network, of very large, macroscopic aggregates. Whether permanently cross-linked or not, the molecular units of the mucus glycoprotein have to overlap or interpenetrate each other sufficiently to form the linked macroscopic network to function mechanically as mucus.

Cross-linking in mucus involves disulfide bonds, physical entanglements[12] and secondary bondings. The large number of cysteines in the non-glycosylated region allows the formation of intermolecular connections between one or more mucin molecules to form long, linear multimer chains. These chains may be woven together forming a randomly entangled mucus network[44-48] as shown in figure 3. These entanglements are

Figure 3: A schematic representation of a randomly entangled mucus network


"NAKED" PROTEIN REGIONS
HEAVILY GLYCOSYLATED REGIONS
OF GLYCOPROTEIN

almost equivalent to permanent cross-links.[12] Branching, however, is essentially covalent in nature, and may substantially influence both conformation and interactions.[40]

Allen et al.[35,49,50] reported that native pig gastric mucus glycoprotein has a molecular weight of $2x10^6$. When reduced by mercaptoethanol, or proteolysed by pepsin, the native glycoproteins were depolymerized into four identical subunits with molecular weight of $5x10^5$, which is equal to a quarter of the original molecular weight. Further proteolysis or reduction has no additional effect on size. Thus, the disulfide bridges are important in attachment of the subunits.[50-52]

Based on the structure of mucin, there are four characteristics of the mucus layer that relate to mucoadhesion: it is a network of linear, flexible, and random coil mucin molecules; it is negatively charged due to the presence of sialic acid, which has a pK_a of 2.6,[53] and sulfate residues on the mucin molecule; it is a cross-linked network, because of disulfide bonds and physical entanglement between mucin molecules;[22,35] and it is also highly hydrated.[11]

3. Physicochemical Properties of Mucus

a. Thickness

The thickness of the mucus layer is the distance between the solution interface and the mucus-mucosa

interface. In-vivo, mucus gel forms a thin but continuous cover of variable thickness over the mucosa. The thickness of the mucus layer varies considerably with organ and species, and can be observed directly on thick unfixed mucosal sections.[51] Thus, the gastrointestinal mucus thickness ranges from 5μm to 200μm, and occasionally up to 500μm, in the rat, and is about two-fold greater in humans. The mean thickness is 192μm in the human stomach, 77μm in the rat stomach, and 81μm in the rat duodenum.[51] In the eye it is 1.4μm on the human conjunctiva.[54]

Luminal pepsin rapidly dissolves the mucus cover. but its continuity is maintained by fresh mucus secretion.[51] Carbenoxolone,[55] prostaglandins, and carbachol[51] increase mucus thickness. Bile, HCl, NaCl (2M), and ethanol (40 or 100%) do not destroy the mucus gel structure.[51] However, by dipping the mucosa into N-acetyl cysteine for 3 minutes the mucus gel thickness is reduced dramatically from 140μm to 65μm.[56] It is thought that continuity of the mucus layer is a critical factor in its protective function. The greater the depth of the gel layer on the mucosal surface of the stomach, the better it will resist total removal by physical abrasion, or penetration by pepsin.[57] The depth of the gel layer will depend on the difference between the rate of secretion of the mucus and the rate of removal by physical or chemical insult. Thus, in studying changes in mucus in-vivo,

both mucus erosion and mucus secretion should be considered.[50,58]

b. Charges

At the terminal ends of all the oligosaccharide side chains of the mucin network, the terminal carbohydrates are either sialic acid or L-fucose.[21,31,32] Sulfate, when present, is linked to either galactose or N-acetylglucosamine.[60] Since the pK of sialic acid is 2.6,[53] it is completely ionized at physiological pH of 7.4. Together with the presence of sulfate residues, the mucin network can be predicted to carry a substantial negative charge at pH7.4. Studies of carbohydrate at the cell surface have suggested that oligosaccharide containing sialic acid is probably located at the outermost portion of various types of cells.[59,61-67,100] It was suggested by Quinton et al.[68] that fixed anionic sites cross-linked with membrane-fixed cationic sites, coordinate with metallic cations and form hydrogen-bonds with other electronegative centers. Thus, epithelial anionic sites might be partly responsible for holding the mucus layer close to the underlying mucosa. Similarly, anionic sites on the mucin network might play an important role in mucoadhesion.

c. Rheology and Gel-forming Properties of Mucus

At physiological pH and ionic strength, the glycoprotein organizes water in its vicinity efficiently and is capable of a wide range of rheological behavior and gel

formation.[69] Mucus has strong attachment properties not only to itself but to other bioadhesive molecules and firmly binds to the epithelial cell surface as a continuous gel layer over the mucosa. Such gels flow and behave as non-Newtonian fluids.[70]

Rheological studies show that mucus is a water-insoluble, viscoelastic gel created by the cross-linked polymer structure.[50,71-73] Since the gel structure collapses on proteolysis with pepsin or pronase, or on reduction with 0.2M mercaptoethanol, the subunits alone do not form a gel.[50] The viscoelasticity of the gel can be restored if non-covalent bond-breaking agents, e.g., guanidine or thiocyanate, are used to dissolve the mucus, whereas breaking of covalent bonds by reducing agents such as mercaptoethanol results in irreversible loss of the gel structure.[74]

It has been observed that mucus does not swell back to its original volume after it has been dehydrated. This may be due to an increase in the degree of cross-linking as the concentration increases.[12] The one difference observed between the native mucus gel and the gel reconstituted from purified gastric mucus glycoprotein is that the latter will dissolve in excess solvent although it will not dissolve if left at 4^{o}C over a period of time.[57] Thus, native mucus gel has a more ordered structure, which is optimal for non-covalent interactions. Thus, many cross-linkages are not covalent bonds, but weak interactions that can be broken by purification, e.g., hydrogen-bonding, electrostatic

interactions, hydrophobic interactions, and physical entanglement.

Gel formation is by intermolecular interaction. The native mucus glycoprotein must exceed a threshold concentration of 30-50mg/ml,[50,75] and gastric mucus glycoprotein has to be concentrated to about 50mg/ml before the interactions which produce gel elasticity occur.[35] When the concentration increases, the linear flexible chains interpenetrate. Molecular entanglement increases as a function of the size and shape of the molecule and the viscosity increases, until a gel forms. In addition, repulsion between the negative charge keeps mucin molecules in an extended configuration, thus enhancing intermolecular interactions.

The weight of carbohydrate varies considerably with the source but does not seem to influence the rheological features of mucus.[12] Perhaps, the role of the carbohydrate in gel formation is its contributions to hydrophilicity.[57] Gastric mucus contains less sialic acid than does cervical[50,76] and intestinal[57] mucin, but the presence of sialic acid over the glycosylated region was found to have no influence on mechanical properties.[77-79] Removal of sialic acid does not influence rheological properties of the mucus[80] and the mucociliary transport for cervical[77] and respiratory[71] mucus. The difference in functional response is related to the amount of mucus glycoprotein present in a unit volume. The concentration of glycoprotein in the mucus may be

the most important parameter determining rheological properties of the mucin.[12] Mucin depends for its action on its capacity to immobilize water, and glycoprotein is responsible for this interaction with water.[43] From permeability data, the glycoprotein is found to be homogeneously distributed.[81]

Litt[69] proposed that mucus is present to organize water in the vicinity of the mucosa. Since glycoproteins and other proteins in the mucus are polyions, mucus is capable of swelling, the extent of which depends on the pH and ionic strength of the hydrating medium.[14,82] Small variations in the amount of water in mucus can produce marked changes in its rheological properties.[83] Moreover, in cervical mucin, calcium concentration appears to regulate the change from viscous to visco-elastic character.[43] Thus, the rheological properties of mucus may be regulated by hydration via control of the transepithelial movement of water, ions and soluble proteins.[14] A higher viscosity for gastric glycoprotein was observed at lower ionic strength (below 10mM).[57] The increase in viscosity is due to a decrease in counterions to shield the negatively charged residues.[57] When shielding by cations decreased, the negatively charged groups, e.g., sialic acid, sulfate and the acidic amino acids, will repel each other with expansion of the network and enhancement of intermolecular interaction of the glycoprotein molecules. However, the rheological properties of mucus gels were essentially unchanged by exposure to the following agents for 18

hours at 25°C : 0.15M or 2M NaCl, HCl at pH2, pig gallbladder bile, 20mM sodium taurocholate, 20mM sodium glycocholate, 8M urea and 6M guanidinium chloride.[51]

The conformational model for the mucus polymer network predicts that variation in the rheological properties of mucus should depend on one or more of the following:[14] degree of entanglement between glycoprotein chains; variation in the stiffness of the glycoprotein chains; and amount of interactions between the glycoprotein network and the water medium. In such a weak gel, the intermolecular linkages cannot be permanent, but must be capable of breakage and reformation with a half-life of the order of seconds for slow flow to occur.[43]

d. pH Gradient of the Mucus Layer

The pH gradient across the mucus layer adherent to the gastric mucosa was first postulated by Heatley,[84] and later supported by Ross et al.[85,86] and Williams et al. using antimony microelectrodes.[87]

(i). Stomach

In the stomach, the gastric mucosa is protected by a combination of several mechanisms: the mucus layer; bicarbonate secretion; mucosal blood flow; and reconstitution of the epithelium after mucosal damage. The bicarbonate secretion by the gastric mucosa is stimulated by prostaglandin,[88] and inhibited by aspirin,[88] and bile salts.[89]

The pH at the epithelial surface of the stomach is approximately 7 when the luminal pH is 2.[90-92] The glycoprotein matrix in the mucus effectively prevents pepsin and other large molecules from passing through, and hydrogen ion was found to diffuse 3-4 times slower than through a similar thickness of unstirred water.[91,92]

Human stomach secretes alkali at a rate of about 500μM/hr under basal conditions, that is at about 10 to 20% of the basal acid secretion rate,[91,92] thus the amount of alkali produced would not be enough to neutralize all the acid. It appears that mucus may act by preventing mixing of bicarbonate with hydrogen ion[11,93] rather than by inhibiting diffusion of these ions. Alkali secreted into a zone of limited mixing provided by the mucus gel might be able to slowly neutralize the acid. Thus, a pH gradient could be created across the mucus layer.[93] Additional hydrogen ions may be eliminated by a controlled amount of back diffusion into the cells where they will be neutralized by an intracellular mechanism.[94]

The pH gradient was demonstrated to depend on integrity of the mucus layer, since addition of N-acetyl cysteine caused a fall in the intermucus pH from 6.68 to 4.18.[95] Thus, it is likely that the mucus layer has some protective role against luminal acid.

(ii). Small Intestine

In the duodenum, the rate of bicarbonate secretion is two to six fold that of the stomach.[96] Acidification of

the luminal solution markedly stimulated epithelial alkaline secretion.[86] The effective pH in the immediate vicinity of the luminal cell surface remained at around neutrality when the luminal pH changed from 7.6 to 2.0[86] or from 4 to 10.5.[97] The pH of the microclimate in the small intestine was 5.7, when pH of the incubation medium was 7.1.[98,99] The thickness of this low-pH compartment at the surface of the intestine was estimated to be 700μm.[141] The acid microclimate is established by secretion of hydrogen ions, rather than bicarbonate absorption, by the epithelial cells in the proximal small bowel, both in-vivo and in-vitro.[101-103]

(iii) Large Intestine

The thin layer adjacent to the mucosa was found to have an approximate pH of 6.5.[104,105] Mucosal disease of the large intestine did not affect surface pH, in contrast to the small intestine where both coeliac and Crohn's disease result in impaired acidification of the microclimate.[106] The amount of mucosal function necessary to maintain a pH of 6.5 is less than the amount that would be needed to maintain one of 5.5, as found in the small intestine, and it appears that this is within the capacity of inflamed large intestinal mucosa.[107] In summary, the large intestine maintains a region whose pH changes much less than luminal pH.

4. Function of Mucus

Mucus in different body locations has different functions (table 1[69]), however, protection is commonly the primary role of mucus.[11] Mucus will provide a stable continuous, unstirred layer over the mucosal surface, and protection is achieved by its antibacterial, and antiviral action, clearance of debris, lubrication, hydration and selective permeability (ion filtration).

Larger particles are held by mucus to be ultimately removed by lateral transport. Although the intestinal mucus layer traps microorganisms,[108] its bacteriostatic action is via non-mucin components.[109] For example : secretory IgA antibody probably acts by blocking attachment of pathogenic microorganisms to mucosal cells; lysozyme associates strongly with mucus glycoprotein to act as a powerful bactericide; and lactoferrin exerts a bacteriostatic action on iron-requiring microorganisms.

Mechanical damage is prevented by lubrication of the surface of epithelial cells and, in this regard, the terminal sialic acid is suggested to play an important role.[108] The optimum mucin concentration for effective lubrication to permit ciliary propulsion in tubes is found to range between 1.7 to 4.1%.[110] In the case of some dry eye diseases, it is believed that mucin loses its wetting ability and forms 'mucin plugs'.

In the gastrointestinal tract, gastric mucin, together with bicarbonate secretion, provides a diffusion barrier for hydrogen

TABLE 1: Types and suggested functions of normal mammalian epithelial mucus (From reference 69)

Type of secretion	Functions
Respiratory	Clearance of mucosal insults; Maintenance of water balance in airway; Maintenance of water balance in mucosa; Ion transport and regulation
Middle ear	Clearance of cellular debris? Water balance
Salivary	Lubrication Water balance Antimicrobial Immunochemical complex
Gastrointestinal	Mechanical protection Lubrication Mixing barrier Diffusion barrier Water balance
Cervical	Regulation of sperm transport Sperm reservoir Antimicrobial Sperm energy source Sperm selectivity Water balance

ion, and maintains a near-neutral pH at the mucosal surface. Gastric mucus also maintains a permeability barrier to pepsin to prevent the underlying epithelium from being damaged. Both in-vivo and in-vitro studies showed that the mucus gel can resist damage from the following agents: 60 or 100% ethanol, 0.6M HCl, 30mg/kg indomethacin, and 80mM taurocholate.[51] Furthermore the hydrophobic mucosal lining of the stomach due to lipid binding may contribute to the ability of the mucus gel to resist back diffusion of hydrochloric acid from the lumen to the epithelial surface.[111] The effectiveness of the mucus gel as a protective barrier will depend on its structural stability as well as its continuity. The polymeric structure of the glycoprotein component is critical for formation of the gel.[51]

Cervical mucus is well known for its cyclic variation in viscosity. When estrogen dominates, there is 0.7-1.5ml of clear mucus with low viscoelasticity which is readily penetrated by sperm.[112] Whereas when progesterone dominates, there is less than 200µl of thick mucus, with high viscoelasticity, that resists sperm penetration.[112] It was concluded that mucus is the essential mechanical coupler transforming the action of cilia into an effective transport system.[113] Other substances which are chemically unrelated to mucus, such as gelatin, agarose, guaran, and acrylamide gels, were also found to be able to couple with the cilia and thus substitute for mucus in its transport role.[113] The only common feature that these polymers have with mucin is that

they are all lightly cross-linked. Thus, light cross-linking density appears to be a basic requirement for a mucus-like substance. The major function common to all mucus secretions is to maintain and control the water balance of epithelial mucosa.[69]

B. Some Proposed Theories of Mucoadhesion

The process of mucoadhesion can be visualized as having two stages: first, establishment of intimate contact; and second, formation of bonds. In the first stage, the establishment of contact by viscoelastic deformation between the bioadhesive and substrate is of primary importance. Thus, the surface characteristics and the composition of the mucoadhesive and mucin, and the applied force or pressure are important parameters. The second stage of mucoadhesion involves interfacial bond formation after contact is established. This interfacial bonding occurs primarily through secondary bonding, e.g., electrostatic and hydrophobic interactions, hydrogen bonding, and van der Waals intermolecular interactions.

For mucoadhesives with charged groups, electrostatic interaction and hydrogen bonding appear to be of primary importance. Electrostatic bonds are caused by coulombic attraction between ions of opposite net charges. Two surfaces, although negatively charged, may attract each other by long-range forces created by atomic and molecular vibrations that produce fluctuating dipoles of similar frequencies on each surface.

The strength of the hydrogen bonding lies between that of van der Waals and primary bonds. Dipping nylon cord into a complex adhesive mixture of rubber and resorcinol-formaldehyde results in absorption or adsorption of the resorcinol-formaldehyde onto

the nylon surface via hydrogen bonds through the phenolic groups.[103] The autohesion of polymers which have been subjected to surface oxidation by immersion in acids[114,115] or exposure to an electrical discharge[116] also reveal the importance of hydrogen bonding.

There are five proposed theories of adhesion which may be adapted to bioadhesion:[9]

1. Wetting

One of the factors that influences adhesive joint strength is the ability of the adhesive to spread spontaneously on the substrate. According to the Dupre' equation,[117]

$$W_{AB} = \gamma_A + \gamma_B - \gamma_{AB} \qquad (1)$$

where W_{AB} is the thermodynamic work of adhesion, γ_A and γ_B are the surface tensions, and γ_{AB} is the interfacial tension. The interfacial energies are responsible for the contact and adhesive strength of the two surfaces. The relationship between contact angle, Θ, and interfacial tension can be demonstrated by the Young equation[117]

$$\cos\Theta = (\gamma_{SV} - \gamma_{SL}) / \gamma_{LV} \qquad (2)$$

The interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities have been studied, and optimal adhesion of human endothelial cells was generally

found with moderately wettable polymers.[118] Within a series of cellulose polymers cell adhesion increased with increasing contact angle of the polymer surfaces. Thus, proper surface treatment is important to establish a desirable strength of bioadhesion.[119]

The interfacial properties of two immiscible polymers were studied using mean field theory.[120-122] The interfacial tension was predicted to be proportional to the square root of the polymer-polymer interaction parameter.[120-122] Structural similarities of the two polymers corresponds to a low value of the polymer-polymer interaction parameter, which means a small interfacial tension, and a subsequent high value of work of adhesion.

2. Diffusion

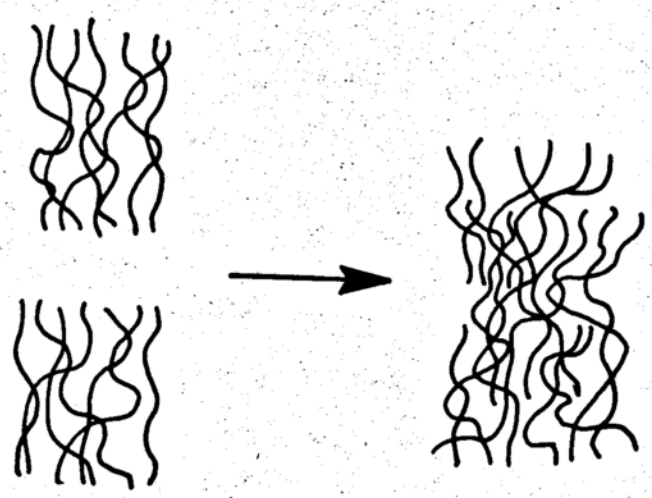
The diffusion theory of adhesion was first discussed by Voyutskii.[123] In this theory, the chains of the adhesive and substrate interpenetrate each other to a sufficient depth and create a semi-permanent adhesive-bond (figure 4). Direct evidence for interdiffusion in compatible polymers does exist and has been demonstrated by radiometric studies.[124]

The exact depth of interpenetration or entanglement needed to achieve adequate bioadhesion is not known, but the representative mean diffusional path, s, for macromolecules can be estimated as[125]

$$s = (2tD)^{1/2} \qquad (3)$$

where D is the diffusion coefficient. For high molecular weight

Figure 4: Schematic representation of interdiffusion (interpenetration) of two polymer solutions



(10^4 to 10^6) polymers, autoadhesion self-diffusion coefficient of 10^{-11} to 10^{-14} cm^2/sec have been observed.[125] Thus, by using equation 3, the representative mean diffusional path for bioadhesion of macromolecules at one second can be estimated to be at roughly 2 to 45 Å. For a contact time of 60 seconds, the estimated mean diffusional path will be 11 to 346 Å.

3. Electronic[126,127]

Adhesives and glycoprotein networks have different electronic structures. When two surfaces come into contact, electron transfer is likely to occur. Subsequently a double layer of electrical charge forms at the adhesive interface, and adhesion occurs due to attractive forces across the electrical double layer. Thus, the adhesive/substrate system is treated as a capacitor, which is charged when two different surfaces come in contact and discharged when they are separated.

4. Fracture

The fracture theory of adhesion is related to the detachment of two surfaces after adhesion. Equation 4 is used to calculate the fracture strength, σ,

$$\sigma = (E\varepsilon/L)^{1/2} \qquad (4)$$

where E is Young's modulus of elasticity, ε is the fracture energy, and L is the critical crack length upon separation of the two surfaces. The work of fracture , G_c, of an elastomeric network

increases with molecular weight, M_c, of the network strands[128-130]

$$G_c = K(M_c)^{1/2} \qquad (5)$$

where K is a constant relating to the density of the polymer, the effective mass, length and flexibility of a single main-chain bond, and the bond dissociation energy. As predicted by Lake and Thomas the work of fracture is greater when the network strands are longer,[128] or the degree of cross-linking smaller.[130]

The mechanical strength of a molecular network formed by the physical entanglement of elastomeric macromolecules has been measured.[131] It was concluded that the work of rupture across the plane of entanglement is roughly proportional to the inferred density of entanglement.[131] The greater the molecular weight of the strands comprising the loops, the greater appears to be the work of rupture.[131]

5. Adsorption

Adsorption theory has been investigated and discussed in depth by Kinloch[132] and Huntsberger.[133] According to this theory, a bioadhesive polymer adheres to tissue because of van der Waals forces, and hydrogen bonding.[134,135] Norde and Lyklema[136-138] found that adsorption occurs when both protein and polystyrene surfaces are negatively charged and the maximum amount adsorbed increases with increasing density of the negative charge at the polystyrene lattice surface. Upon

adsorption, the electric fields of the protein molecule and the adsorbent surface overlap each other, involving redistribution of charge and charge transfer.

The interfacial bonding force at the contact surface of polystyrene is primarily due to London dispersion forces, e.g., van der Waals forces.[139] These forces exist between the repeat units of the polymer on the two sides of the interface.[139] At solid-liquid interfaces, almost all absorbed macromolecules were found to form monolayers, with the layer thickness varying with the square root of the molecular weight.[140] The formation of adsorption bonds on the interface depends greatly on the properties of the macromolecule: molecular weight; chemical structure; flexibility of the chain-segment; and charge density.

C. Bioadhesive Polymers/Copolymers

Bioadhesives used or tested to date are all polymers of high molecular weight. The molecular weight distribution of soluble polymers can be determined by chemical or physical methods, e.g., end-group analysis, colligative properties, light scattering, ultracentrifugation, and dilute solution viscosity measurement.[142]

Except for natural polymers, polymers are usually synthesized by polymerization of monomers, with or without cross-linking agents to give linear or cross-linked (insoluble) polymers, respectively. If more than one kind of monomer is involved, the polymer obtained is called a copolymer. The

desirable features of a polymer can be controlled by varying the type and amount of monomers, and/or cross-linking agents.

1. Molecular Characteristics

a. Cross-linking agent

For all water-swellable but insoluble polymers, the linear chains are connnected via cross-linking agents, as in polycarbophil, which is polyacrylic acid cross-linked with divinyl glycol. A series of cross-linked, swellable polymers structurally related to polycarbophil were synthesized and their mucoadhesive strengths evaluated.[6] It was found that there is no significant difference in mucoadhesive strength of three polyacrylic acid polymers with different cross-linking agents. Methyl substitution on the polyacrylic acid backbone, however, reduced mucoadhesion significantly.[6] Since values of the diffusion coefficient decreased with the presence of side-groups and with increasing diffusant size and molecular weight,[125] the reduction in mucoadhesion might be due to an increase in hydrophobicity with a subsequent decrease in hydration of the network, and flexibility and mobility of the segment chains. Thus, the polymer backbone, or the monomeric units, has a more important role in mucoadhesion than the cross-linking agent, because the cross-linking agent is only a very small part (0.2% by weight) of the copolymer.

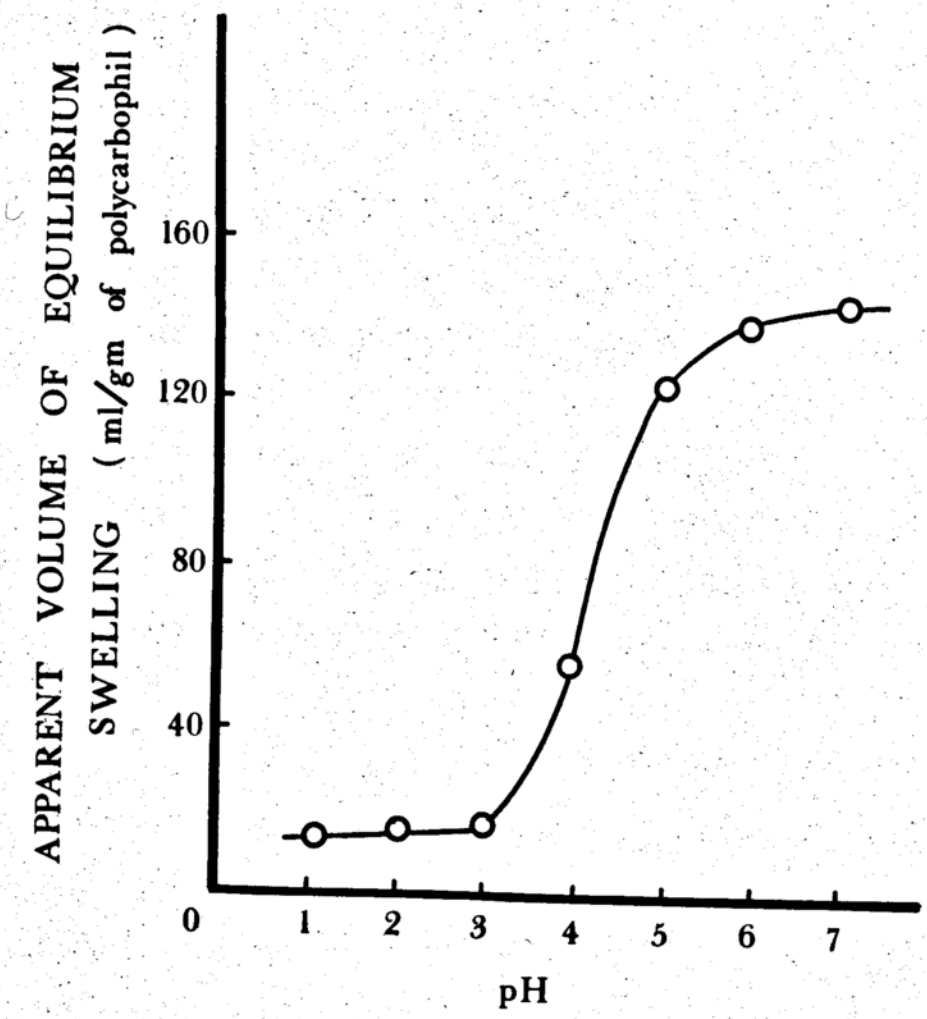
b. Hydrophilic Functional Groups

Park et al.[5] measured and compared the

adhesiveness of a series of anionic, cationic and neutral polymers using a cell culture-fluorescent probe technique. The authors[5] concluded that the charge sign is a very important element for bioadhesiveness of polymers. Polyanionic polymers are preferred over polycationic and neutral polymers when both bioadhesiveness and toxicity were considered. Bioadhesive polymers are usually macromolecular organic hydrocolloids with numerous hydrophilic functional groups that can form hydrogen-bonds,[2] e.g., carboxyl, hydroxyl, amide, and sulfate groups. Hydrogen-bonding appears to play a significant role in bioadhesion, and the presence of water seems to be a prerequisite for a majority of the bioadhesive phenomena. It was found that the degree of hydration of cross-linked polyacrylic acid with carboxyl groups can be controlled by adjusting the pH of the medium (figure 5).[1]

For a macromolecule with charges, in order to satisfy the requirement that the solution of macro-ions must be electrically neutral, the solution must always contain a sufficient number of small ions to neutralize the macro-ion charge.[143] Thus, the Donnan effect cannot be neglected. For a macromolecule in solution, the charge of the macro-ion is compensated for by a change in concentration of small ions. The polymer acts as a membrane preventing the randomly distributed charges from diffusing into the outer bulk solution. Because of the attraction of these fixed charged substituents, the concentration of mobile ions

Figure 5: Apparent volume of equilibrium swelling of polycarbophil at various pHs (From reference 1)


APPARENT VOLUME OF EQUILIBRIUM SWELLING (ml/gm of polycarbophil)
160
120
80
40
0
1
2
3
4
5
6
7
pH

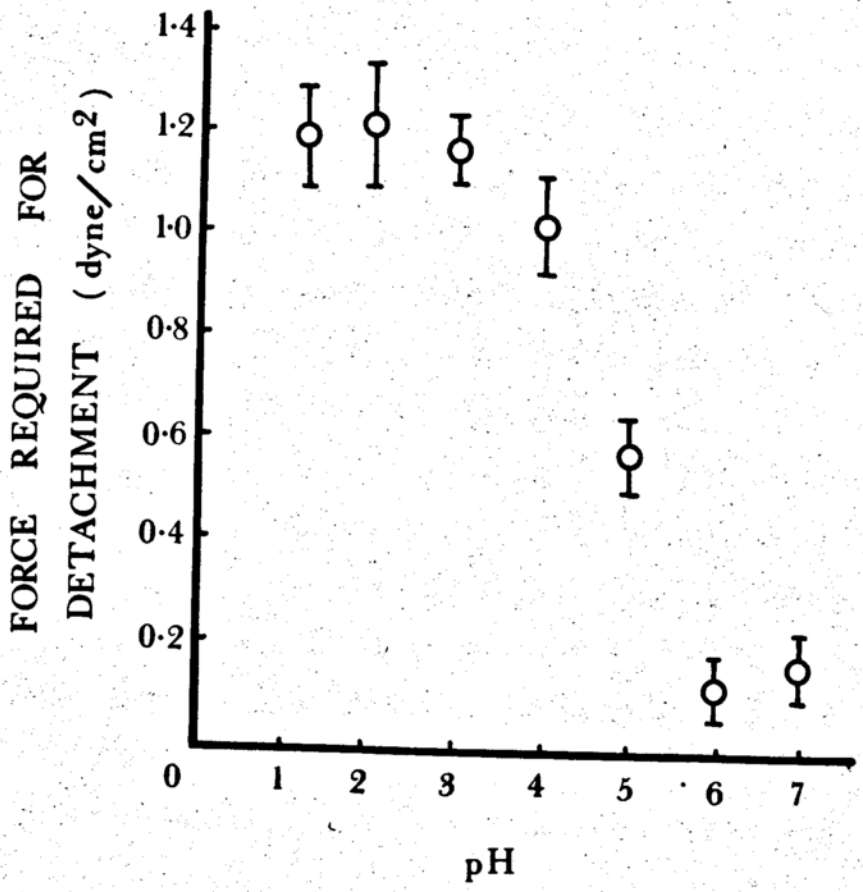
inside the gel will always be greater than those on the outside. Thus, the presence of these fixed charges results in an establishment of a swelling force, or the swelling pressure, or a net osmotic pressure across the membrane.[144] This difference in osmotic pressure will be the driving force for solvent to enter the gel from the more dilute external bulk solution.

For anionic and cationic polymers, e.g., polyacrylic acid and polylysine, the charged groups along the polymer chains establish an electrostatic repulsion which tends to expand the network. However, this electrostatic repulsion can be reduced tremendously by the screening effect of counterions. Thus, the ionic strength, and the concentration of counterions in solution, is an important parameter that has to be considered in bioadhesive studies.

In figure 6,[189] the adhesive strength of cross-linked polyacrylic acid, pK ≅ 4.5, is shown as a function of media pH. The adhesive force drops sharply at around pH4, which suggests that mucoadhesion is highly favored when carboxyl groups are in an acid form. Thus, the interaction in an adhesive-mucin interface is suggested to occur through hydrogen-bonding.

Polyacrylic acid (pK = 4.75) is a stronger acid than polymethacrylic acid (pK =5.65), and each polymer is a weaker acid than the corresponding monomer (pK of acrylic acid is 4.26 and pK of methacrylic acid is 4.66).[188] The pK of polyacrylic acid was found to depend on the degree of neutralization. At 50%

Figure 6: Effect of pH on in-vitro bioadhesion of cross-linked polyacrylic acid to rabbit's stomach tissue (From reference 189)


FORCE REQUIRED FOR DETACHMENT (dyne/cm²)
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
1
2
3
4
5
6
7
pH

neutralization, the pK of polyacrylic acid was 5.20.[189] It is likely that cross-linked polyacrylic acid may have maximum mucoadhesion at a pH less than 4, where the majority of carboxyl groups are non-ionized and available for formation of hydrogen bonds. For cross-linked polymethacrylic acid, the pH range for maximum mucoadhesion may be higher than that of cross-linked polyacrylic acid by one pH unit. Thus, depending on the pK of the mucoadhesive, the mucoadhesive strength can be maximized by controlling the degree of ionization of the charged groups via manipulation of the madium pH.

c. Molecular Weight, Chain Length, and Conformation

Chen and Cyr[2] suggested that bioadhesive strength increases as the molecular weight of polymer increases above 100,000. For example, polyethylene glycols, polymers with a highly linear configuration, with molecular weight of 20,000 (Carbowax 20M) have no wet adhesive property, whereas, at a molecular weight of 200,000 (polyvox WSR 35) adhesiveness is improved, and an excellent adhesive is obtained if the molecular weight is increased up to 4,000,000 (polyvox WSR 301). It was found that degraded carrageenan is much less effective as an adhesive than the higher molecular weight potassium carrageenate.[2] Thus, chain length may be an important parameter that controls bioadhesive strength. Bioadhesives should thus be protected from chemical or bacterial degradation

during storage to prevent chain length shortening.

Dextrans of molecular weight as high as 19,500,000 have similar bioadhesive strength to those with molecular weights of 200,000.[145] One possible explanation is that the dextran molecule is in a helical conformation, which is different from the linear conformation of polyethlene glycols, and which would shield many of the adhesively-active groups inside the coils. For polyethylene glycols, the increase in molecular weight would increase the chain length, which in turn favors penetration and interaction of the polymer with the substrate. It was found that the molecular weight of sodium carboxymethyl cellulose (NaCMC), a potential bioadhesive, should exceed 78,600 in order to have significant bioadhesion.[146] It was proposed that a critical molecular length exists in bioadhesive systems, which influences bioadhesion via its effects on interpenetration and entanglement of the polymer to the substrate.[146] Thus, molecular weight, chain length and conformation of the macromolecule are all important parameters that affect bioadhesion.

d. Molecular Flexibility

Bioadhesive polymers are usually macromolecular hydrocolloids with numerous hydrophilic groups, possessing swelling and hydration properties. When they are placed in an aqueous medium, they swell and expand into a gel or network. Since interpenetration and entanglement of the bioadhesive polymers to the substrate are proposed to be partly

responsible for their bioadhesive properties,[146] mobility, and flexibility of the polymer chains is one of the important variables that must be considered.

The ability of the polymer chains to interpenetrate can be approximated by their ability to diffuse. Thus, chain segment mobility of the polymers and substrates can be related to their viscosities and diffusion coefficients. The diffusion coefficient of the solute increases with degree of swelling of the network, and decreases with increasing solute size.[147] Over sufficiently restricted temperature ranges, the experimental diffusion coefficient, D, shows an exponential temperature dependence of the Arrhenius type[147]

$$D = D_0 \exp(-E/RT) \qquad (6)$$

where the pre-exponential factor D_0 is a constant and is independent of temperature over a given temperature range, and E is the experimental activation energy for diffusion or for mobility of the segment chains. Thus, the higher the chain segment mobility at a particular temperature, the greater the interdiffusion. Rigidity of the repeating units on the polymer chain will affect the energy of mobility. If they are stiff, then it is more difficult to move and diffuse into the substrate.

Another factor that will affect diffusion and the interpenetration process is the number and size of pores in the hydrated network. It is assumed that solute diffusion occurs only

through solvent regions and that the macromolecular chains of the network can be forced to a non-equilibrium state by the diffusing solute.[148] The term 'pore' is a little misleading; a more appropriate term for the available space in the networks is 'macromolecular mesh'.[149] In a swollen macromolecular network, the effective mesh size, ξ, or the effective area for diffusion, is the space between the spheres where the chains are confined. Critical hole formation occurs by increased chain flexibility and chain movement.[125] The average mesh size, $\overline{\xi}$, the number average molecular weight between cross-links, $\overline{M}_c$, and the degree of cross-linking, are three interrelated structural parameters of a polymer network.

The average mesh size of the network structure in angstroms can be represented as[150]

$$\overline{\xi} = \alpha_s (2nl^2)^{1/2} \qquad (7)$$

where l is the bond length, n is the number of links between two cross-links, and

$$\alpha_s = (V/V_o)^{1/3} \qquad (8)$$

where V is the volume of the swollen polymer, and V_o is the volume of the unswollen polymer. Experimental determination of mesh size has been reported using neutron scattering[151] and laser light scattering techniques.[152]

2. Physicochemical Features

The physicochemical features of mucoadhesive polymers depend to a large extent on the monomer units and on the way the monomers are put together.

a. Viscosity

One of the most important properties of a bioadhesive polymer is its aqueous viscosity, which is caused by polymer-solvent interaction and depends on the flexibility of molecules, the type of solvent, the degree of ionization, the concentration of polymer, and pH.[153] The viscosity of most polymers does not have a single value but varies with the shearing force of the flow.[154] There is a proportional relationship between the shear force per unit area, σ_s, and velocity gradient, $\Delta v/\Delta y$[154]

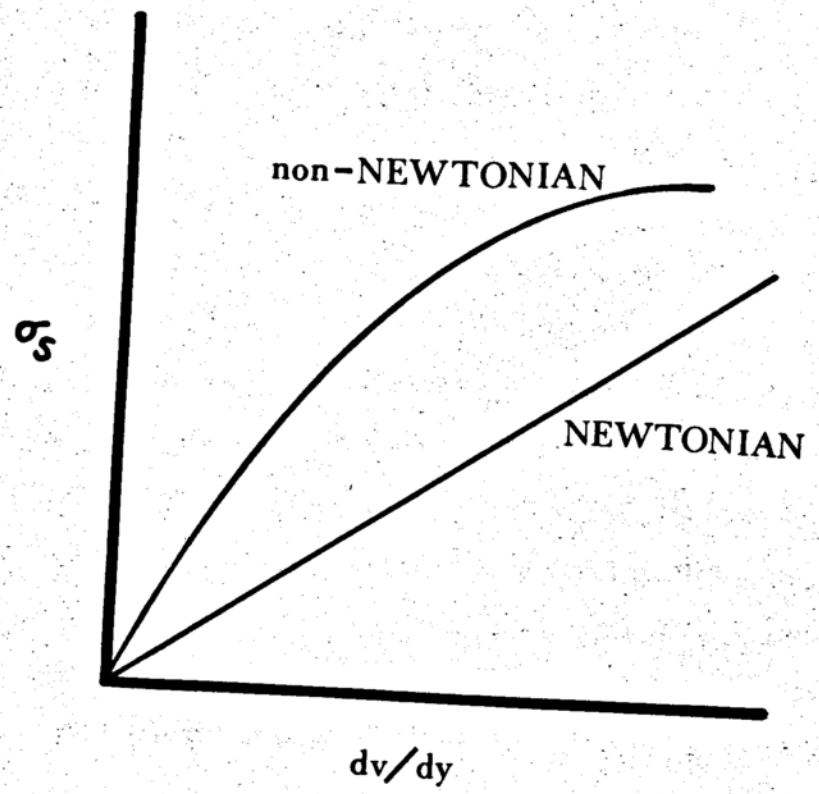
$$\sigma_s = \eta \, \Delta v/\Delta y \qquad (9)$$

where η is the coefficient of viscosity, or "viscosity", of the fluid. Equation (9) can be written as

$$\sigma_s = \eta \, dv/dy \qquad (10)$$

and those systems which obey equation (10) are called Newtonian (figure 7). Solutions of low molecular weight solutes are generally Newtonian, and solutions of macromolecules generally are non-Newtonian. Intuitively, longer chains should experience

Figure 7: Differentiation between Newtonian and non-Newtonian behavior


non-NEWTONIAN
NEWTONIAN
σ_s
dv/dy

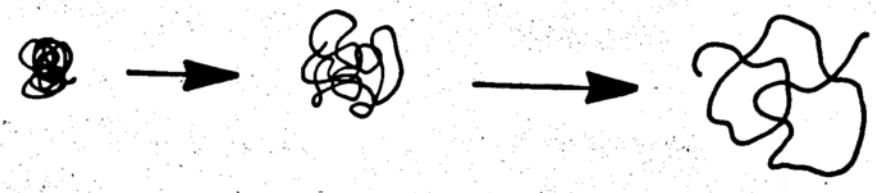
higher resistance to flow and have the ability to attract and entangle with each other; it is understandable then for them to have non-Newtonian behavior.

Viscosity is found to depend on molecular weight.[154] Branched polymers have lower viscosity for the following reasons:[154] first, the backbone chain is shorter for branched than linear polymers of the same molecular weight; second, the branched polymer is more compact.

For the same polymer, its viscosity is affected by the degree of entanglement between different polymer chains, which is higher when the polymer chains are in a more extended form. In a 'good' solvent, polymer-solvent interactions are favored over the polymer chain-chain interactions, thus polymer molecules expand well in this solvent. In a 'poor' solvent, the intermolecular interactions between the polymer segments are greater than the segment-solvent affinity, and the molecular chains would thus tend to be more contracted (figure 8). Two substances that have nearly the same solubility parameter, δ, will be mutually soluble. Thus, a good solvent can be found by looking at the solubility parameter of solvent and polymer. The polymer chain is more extended for a good solvent, thus the intrinsic viscosity of the polymer will be higher in a good solvent.

Most bioadhesive polymers have hydrophilic groups, which interact with water molecules in an aqueous media, and expand the polymer chain network to a freely moving state. When the

Figure 8: Schematic representation of dissolution/ swelling of polymer in good and poor solvents



POOR
SOLVENT

GOOD
SOLVENT

bioadhesive is in contact with a hydrophilic substrate, the stretched and entangled polymer molecules can match their active adhesive sites with those on the substrate to form adhesive bonds. Thus, good water swelling or hydration is a prerequisite for bioadhesion. For charged bioadhesives, the effect of pH on swelling is important.

b. Swelling, Hydration and Gel-forming Properties

Bioadhesion of a polymer to a dissimilar surface can be described as a dynamic equilibrium of making and breaking adhesive bonds between the adhesive and the substrate through the presence of water.[190] When a bioadhesive polymer approaches the substrate surface with functional groups capable of competing with a weak boundary layer, i.e., water, and solidifies to a rigid structure, bioadhesion occurs. This dynamic equilibrium of bonding and debonding in the presence of water allows stress relaxation at the interface.[190] The adhesion of plastics and ice to moist surfaces, barnacles to underwater structures, and plaque to teeth, etc. are examples of adhesion by this mechanism.[190]

In hydration of macromolecules, the water can be divided into three categories:[155] first, tightly bound water, which is thought to be hydrogen-bonded to the macromolecule; second, loosely bound water, which differs from bulk water in enthalpy and/or temperature of freezing; and third, bulk water, which has

normal freezing properties.

When mucoadhesives contact with an aqueous media, they swell and form a gel. The rate and extent of water uptake by the mucoadhesive may be depended on the type and number of hydrophilic groups in the polymer structure, and the pH and ionic strength of the aqueous medium.

Hydrocolloids have different adhesive properties at different degrees of hydration, and maximum adhesion is reached at an optimum degree of hydration.[2] When insufficient water is used to hydrate the bioadhesives, not all the adhesive sites are liberated and exposed for interaction. Furthermore, at insufficient hydration not all polymer chains are mobile and available for interpenetration to occur. On the other hand, when an excessive amount of water is used, an overextension of hydrogen-bonds and other adhesive forces between the substrate and bioadhesive may result in a weakening of adhesive strength.[2] In other words, some hydrocolloids exhibit adhesiveness at a low degree of hydration, and lose some of their adhesiveness when excessive water is added.

For water-insoluble polymers, the three-dimensional network in aqueous medium is maintained by covalent bonds formed during polymerization in their synthesis. In a stretched network structure, the force of retraction depends on the degree of cross-linking. The degree of swelling at equilibrium was found to decrease with increasing degree of cross-linking.[144]

For assessment of bioadhesiveness, the swelling time of the gel is another important factor that needs to be considered. At initiation of swelling, the polymer chains are not fully extended, the segment chains are not at their optimum flexibilility and mobility, and formation of molecular meshes (pores) are not maximized to expose available active adhesive sites. Thus, bioadhesive strength is likely to be small due to incomplete adhesive bond formation, and minimal interpenetration and entanglement. Therefore, the faster the polymer is hydrated, the faster will be the initiation of diffusion, and formation of adhesive bonds, and the faster will be the initiation of bioadhesion.

c. Macromolecular Complexation

(i) Complexation with Divalent Ions, e.g., Calcium

The divalent ion, calcium, is known to precipitate mucin,[156-158] increase its permeability[159] or reduce its viscosity,[158] and bind to other proteins, e.g., fibrinogen,[160] bone mucosubstances,[161] concanavalin A,[162,163] uvomorulin,[164] β–casein,[165] elastin and collagen,[166] a glycine tripeptide,[167] and tubulin.[168] It was also found that the calcium-induced precipitate of phosphoprotein was dissolved by addition of a small amount of EDTA,[157] a known calcium-chelator.

There are two mechainisms by which calcium-induced glycoprotein aggregation may occur.[156] First, calcium can form

bridges between negatively charged groups,[167] e.g., phosphate and carboxyl groups of adjacent monomers or dimers; second, calcium can enhance a conformational change in the glycoprotein by exposing more hydrophobic surfaces for aggregation.[156]

Forstner and Forstner[158] found that sialic acid in the mucin molecule appears to account for about 90% of the bound calcium. Removal of sialic acid by incubating mucin with N-acetyl neuraminidase in acetic acid/KOH buffer abolished the apparent cooperativity of binding.[158] It appears that calcium neutralizes anionic groups (mainly sialic acid), and this permits the flexible threads of mucin to coil or contract into a slightly smaller hydrodynamic volume. Neighboring carboxyl groups were then more capable of sharing calcium, hence cooperativity resulted.[158]

(ii) Complexation with Surfactants

When examining polymer/surfactant association, it is useful to consider the self-association, or micellization, of a typical ionic surfactant, e.g., SDS, as a model. Reduction of the hydrocarbon/water contact area of alkyl chains of the dissolved surfactant is the chief driving force for association.[169] One of the forces resisting association is the crowding of the ionic headgroups at the periphery of the micelle, which favors the tight binding of counterions and diminishes electrical potential and headgroup repulsion.

For a synthetic water-soluble polymer with sufficient chain

flexibility, one can imagine that the macromolecule can have a configuration which allows ion-dipole association between the dipole of the hydrophilic groups and the ionic headgroup of the surfactant, and allows contact between hydrophobic segments of the polymer and the exposed hydrocarbon areas of the micelle. There is a minimum molecular weight required for reacting with the surfactant cluster, and polymers with high molecular weight can loop around several surfactant clusters.[169] Hydrophobicity is expected to influence the polymer-surfactant interaction. High hydrophobicity lowers the molecular weight limit required for interaction.[169]

The association of oppositely charged polyelectrolytes and surfactants is strong because it involves strong forces of electrical attraction, which is the primary driving force for interaction. The electrostatic interaction is enhanced by a cooperative process involving association of the alkyl chains of the bound surfactant molecules.[170] Binding of charged surfactants to polyions is expected to involve a substantial change in the conformation of the macromolecules. In summary, there are several factors that influence polymer-surfactant interactions: density and location of charges; hydrophobicity; and backbone and chain flexibility.

(iii) Complexation with polyelectrolytes

When two kinds of macromolecules are mixed in an aqueous media, a complex may be formed due to intermolecular interaction, e.g., the polycomplexes formed by

mixing polyacids and polyglycols, polyvinylpyrrolidone and polyacrylic acids,[153] and fibronectin and hyaluronic acid.[171] Such reactions are highly selective and strongly dependent on molecular size and conformation.[153]

Polyacrylic acid (PAA) and polymethacrylic acid (PMA),[172] PAA and polyethylene oxide (PEO),[172-176] and DNA and polycations[177] are known to form polycomplexes in aqueous solutions. The molecular complex of PEO and PAA appears to be an association between ether oxygens and carboxyl groups through hydrogen bonding,[172-175] and the complex is destroyed by ionization of PAA.[172] The association is suggested to be a cooperative process,[172,175] and its stability depends on the length of the interacting chains.[175]

Polycations were found to form more stable complexes than their corresponding low molecular weight analogs.[178] Polyions with higher charge density are suggested to have stronger complexes because of their stronger cooperative activity.[177] Furthermore, the association of polymethacrylic acid with polyethylene oxide is stronger than that of PAA with PEO.[173] Thus, hydrophobicity plays a significant role in stability of the polycomplex between macromolecules.[176,178,179]

In conclusion, when studying polycomplex formation between polyions (e.g., mucins, and polyacrylic acid) or a cross-linked polyion network, e.g., cross-linked mucin and cross-linked polymers, one has to consider the following factors:

charge density, hydrogen-bond formation ability, hydrophobicity, chain length and backbone chain flexibility of the macromolecules, and ionic strength and pH of the medium.

D. Statement of the Problem

Bioadhesive polymers and/or copolymers have recently received considerable attention as platforms for controlled drug delivery. In using bioadhesives to prepare dosage forms, it is necessary to consider polymers or copolymers with both good adhesive strength and drug releasing profile. Thus, a better understanding of the structure-property relationship between bioadhesives and the mucin-epithelial layer is essential.

Mucus is a continuous network of cross-linked glycoprotein that covers most of the body orifices and the bladder. At pH7.4, the mucin network carries a substantial negative charge due to the presence of sialic acid and sulfonic acid residues. The negative charge of the mucin network is particularly important for interaction with negatively or positively charged bioadhesives.

Thus, the influence of charge and charge-related properties to mucoadhesion were studied by synthesizing a series of 0.2% cross-linked copolymers (acrylic acid - methyl methacrylate).

The insolubility of cross-linked copolymer networks presents a difficult but challenging task in quantitative characterization of their percent compositions. Two quantitative methods of analysis were used; they were the acridine orange binding technique, and infrared spectrophotometry.

The effect of charge group, i.e., carboxyl groups, on mesh size of the network, degree of hydration, and tensile strength required for separation were studied. The dependence of tensile

strength on degree of hydration, mesh size, diffusional coefficient, time and temperature were evaluated and studied.

There are two different kinds of strengthes involved in mucoadhesion; they are tensile and shear strengthes. In order to better understand mucoadhesion, a dual tensiometer was designed to study shear strength required for separation. The effects of applied weight and the expanded nature of the mucin to the interacting interface were also studied.

When the influence of these parameters on mucoadhesion were studied and evaluated, proper mucoadhesives with good mucoadhesive property can be designed and used in the formulation of mucoadhesive dosage forms.

III. EXPERIMENTAL

A. Equipment

Fluorimetric measurements were made with a Fluorescence Spectrophotometer (MPF-4 by Perkin Elmer). Infrared spectrophotometric measurements were made with an Infrared Spectrophotometer (599B by Perkin Elmer). Relative light intensity measurements in bioluminescent experiments were made with photometer (Chem-Glow® II by SLM AMINCO®).

B. Reagents

Acrylic acid, methyl methacrylate, benzoyl peroxide, magnesium sulfate, trypan blue and cyclohexane (HPLC grade) were obtained from Aldrich Chemical Company (Milwaukee, WI). Methanol, ether and calcium chloride were obtained from Mallinckrodt Inc. (Paris, KY). Ethanol was obtained from U.S. Industrial Chemical Company (New York, NY). Chloroform was obtained from MCB Manufacturing Chemists Inc. (Cincinnati, OH). Phosphoric acid and EDTA were obtained from Fisher Scientific Company (New Jersey). Potassium bromide (infrared grade) and bioluminescent adenosine 5'-triphosphate (ATP) assay kit were obtained from Sigma Chemical Company (St. Louis, MO). Neutral red was obtained from Eastman Organic Chemical (Rochester, NY). All the other chemicals were obtained from Sigma Chemical Company (St. Louis, MO). Acridine orange was obtained from

Sigma Chemical Company (St. Lousi, MO) and purified by the method of Lamm and Neville.[180] Carbopol ® EX-83 and EX-140 were gifts from The BF Goodrich Company (Cleveland, OH). All the other chemical were either analytical or reagent grade and were used as received.

C. Materials

The 15ml graduated polystyrene centrifuge tube with cap was obtained from Corning (Chicago, IL). The Nitrex ® monofilament nylon screen (100 mesh) was obtained from Tetko, Inc. (Elmsford, NY). The Roller-Smith precision balance was obtained from Biolar Corporation (North Grafton, MA). The Barnes model 129 potassium bromide die was obtained from Barnes Analytical (Stanford, CN). And the Agate mortars and pestles were obtained from Wilmad Glass Company, Inc. (Buena, NJ). Male albino rabbits (New Franken Rabbitry, WI) were used in all experiments.

D. Methods

1. Synthesis of a series of cross-linked acrylic acid-methyl methacrylate copolymers

In order to study the contribution of anionic polymer structural features to mucoadhesion, a series of 0.2% cross-linked copolymers (acrylic acid-methyl methacrylate) (x-AA/MM) were synthesized using free-radical

polymerization.[181] The molecular structures of acrylic acid and methyl methacrylate are shown in figure 9 and figure 10 respectively.

One hundred and sixty grams of magnesium sulfate heptahydrate was added to a 500ml 3-neck round bottom flask. Twenty ml of double distilled water was added, and the flask was heated to 95°C while stirring. The temperature of the resulting salt solution was allowed to stabilize. Then 20gm of acrylic acid-methyl methacrylate (in different proportions) mixture, 0.04 gram of divinyl glycol and 0.2gm of benzoyl peroxide were mixed and added to the salt solution. Polymerization occurred within a short period of time (within 5 to 10 minutes). After polymerization, the mixture was maintained at 95°C with stirring for two hours of curing time. At the end of the reaction, the mixture was diluted with 150ml of hot water and strained through a 100 mesh Nitrex® monofilament nylon screen. The copolymers were washed with one liter portions of deionized water. The washed copolymers were then stirred in two liters of deionized water continuously for one week.

The deionized water was changed once daily and the copolymers were washed with two liters of deionized water. The washed copolymers were dried in a hot air oven at 90°C, and then ground with a mortar and pestle to give copolymers of varying particle sizes.

Figure 9: Molecular structure of acrylic acid

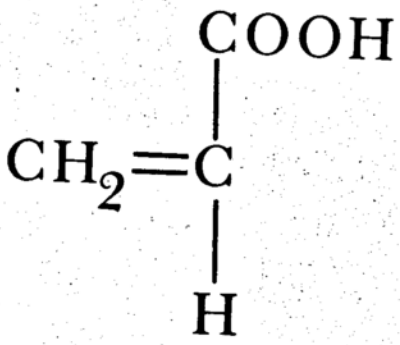
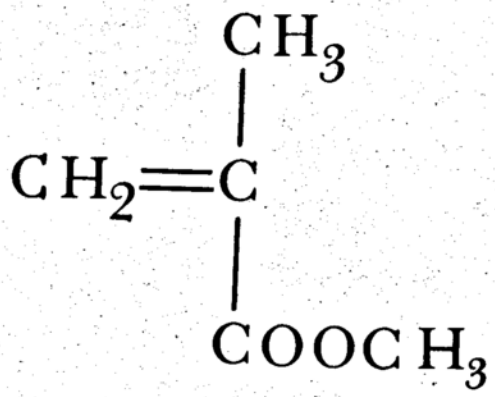

COOH
CH2=C
H

Figure 10: Molecular structure of methyl methacrylate


CH3
CH2=C
COOCH3

2. Quantitative characterization of copolymers

a. Acridine orange binding technique

(i) Fluorimetric measurement

Fluorimetric measurements were made with a Perkin-Elmer MPF-4 fluorescence spectrophotometer. Samples were maintained at 25°C by circulating water through the cell holder. Acridine orange was excited at the short wavelength of the monomer absorption maximum at 400nm. Fluorescence is viewed at 540nm instead of at the maximum intensity of 530nm, in order to minimize inner filter effects from self-absorption.[182,184] A Corning glass filter (number 9863) was used to isolate the wavelength region for excitation, and a cut-off filter (UY 43) was used for emission. The fluorescent emission intensity was determined relative to a sample of unbound dye. Under these conditions, the intensity of fluorescence is directly related to the concentration of free monomer dye.[183]

(ii) Determination of percent composition of acrylic acid in acrylic acid-methyl methacrylate copolymers

Adsorption of acridine orange onto the polystyrene centrifuge tube was done[183] and was concluded that negligible amounts of acridine orange adsorbed onto the polystyrene centrifuge tube during the course of the experiment.

Approximately 100μg of x-AA/MM copolymers of different mesh sizes were carefully weighed and transferred to 15ml polystyrene centrifuge tubes with caps. Fifteen ml of 1 - 2 $x10^{-5}$M acridine orange solution were pipetted into each centrifuge tube. All tubes were wrapped with aluminum foil and vortexed for 10 to 30 seconds. The tubes were then centrifuged at half speed for 5 minutes. Fluorescent intensity of the supernatant was measured and compared with the control. The supernatant was transferred back to the corresponding centrifuge tube and vortexed. Fluorescent intensities of the supernatant were measured at different time points. The procedure was repeated up to 10 hours until there was no further decrease in fluorescent intensity. The experiments were conducted at 25°C. The acridine orange solution was adjusted to pH9 with 0.1N NaOH. At the end of the experiment the pH's of the polymer-dye solutions were measured and found to be around pH 7.5.

b. Infrared spectrophotometry

(i) Preparation of KBr pellets for infrared absorption measurement

Potassium bromide powder (IR grades) was ground using the Agate mortar and pestle. The ground KBr powder was dried in an oven for three days and stored in an vacuum dessicator. One hundred mg of the ground and dried KBr powder was mixed with 1 - 2 % of the copolymer powder (<325 mesh cut) in the Agate mortar and pestle. The

KBr/copolymer mixture was transferred to a Barnes model 129 KBr Die and compressed to form the KBr pellet by using a Carver 12-ton hydraulic press. The pressure used was 10,000 lbs/in^2.

(ii) Preparation of standard curves for cross-linked polyacrylic acid and cross-linked polymethyl methacrylate.

One hundred mg of ground and dried KBr powder was mixed with varying amount (0.5% to 2.5% by weight) of cross-linked polyacrylic acid (x-AA) or cross-linked polymethyl methacrylate (x-MM). The mixture was compressed to form KBr pellets for infrared absorption measurement. The wavenumbers used for x-AA and x-MM were 1725cm^{-1} and 365cm^{-1} respectively. The standard curves were used to quantitate the percent compositions of methyl methacrylate and acrylic acid in the synthesized cross-linked acrylic acid-methyl methacrylate copolymers.

3. Hydration study of the synthesized copolymers

Copolymers with diameter around 5mm were used for hydration study. Hydration of the copolymer particles were done on petri dish using pH2 isotonic phosphate buffer. The amount of water uptake was determined by the difference in weight of the copolymer particles before and after hydration. The amount of water uptake at different time points was recorded until there was no more increase in water uptake.

4. Viability test of rabbit stomach tissue

a. Two-dye method

Male albino rabbit was sacrificed by injecting sodium pentobarbital solution intravenously. The animal stomach was removed and cleaned with chilled normal saline solution and kept in chilled and aerated normal saline solution. At different time points, a small piece of stomach tissue was removed and lightly blotted dry. The tissue was first stained with neutral red for 15 minutes, then with trypan blue for 5 minutes. The tissue was then washed with normal saline carefully two to three times before mounting onto a slide. The colors of the cells were observed and recorded.

b. Bioluminescent method

Analysis of the intracellular concentration of ATP can be used to measure the energy status of cells. ATP is the basic "energy unit" of living cells and is rapidly destroyed by ATPases upon cell death. Thus, cell death is accompanied by a rapid and complete loss of ATP. ATP levels can be used to quantitate the number of viable cells present. The bioluminescence assay provides a simple, rapid and sensitive method for measuring ATP.

(i) Standard curve

ATP standard stock solution was prepared by dissolving the entire content of the ATP standard vial in 10ml

of deionized and filtered water and stored at -20^{0}C. The standard stock solution (10^{-5} g/100µl) is good for up to two weeks at -20^{0}C.

The ATP standards (10^{-9} g/100µl to 10^{-6} g/100µl) were prepared by making serial dilutions of the standard stock solution. The measurement of relative light intensity was the same as in section b(ii).

(ii) Samples

Male albino rabbit was sacrified by injecting pentobarbital solution intravenously. The animal stomach was removed and cleaned with chilled normal saline solution and kept in chilled and aerated normal saline solution. At different time points, small pieces of stomach tissue (50 to 100mg) were removed and lightly blotted dry. The tissue was then placed quickly into ethanol (in ethanal/ dry ice solution). The weight of the tissue was obtained prior to homogenization.

The tissue was placed in a frozen homogenate tube. One-half ml of ice-cold 3N perchloric acid was added and homogenized on ice for one minute. One ml of ice cold 1mM EDTA was then added to the mixture and homogenized on ice for 30 seconds. The sample was centrifuged at 3600 rpm for 15 minutes at 4^{0}C. One ml of supernatant was withdrawn and added to a tube containing 500µl of 2M potassium carbonate and vortexed. The mixture was

then incubated for 10 minutes on ice. Forty µl of 2M TRIS was added and vortexed. The sample was then centrifuged at 3600 rpm for 15 minutes at 4°C. The supernatant was removed for use in the ATP assay.

ATP assay mix stock solution was prepared by dissolving the entire content of the ATP assay mix vial in 5ml of deionized and filtered water and kept in ice for one hour to assume complete dissolution. The ATP assay mix stock solution was then stored in small aliquots at -20°C in the dark. The assay mix for experiment was prepared by diluting the stock solution 125 fold.

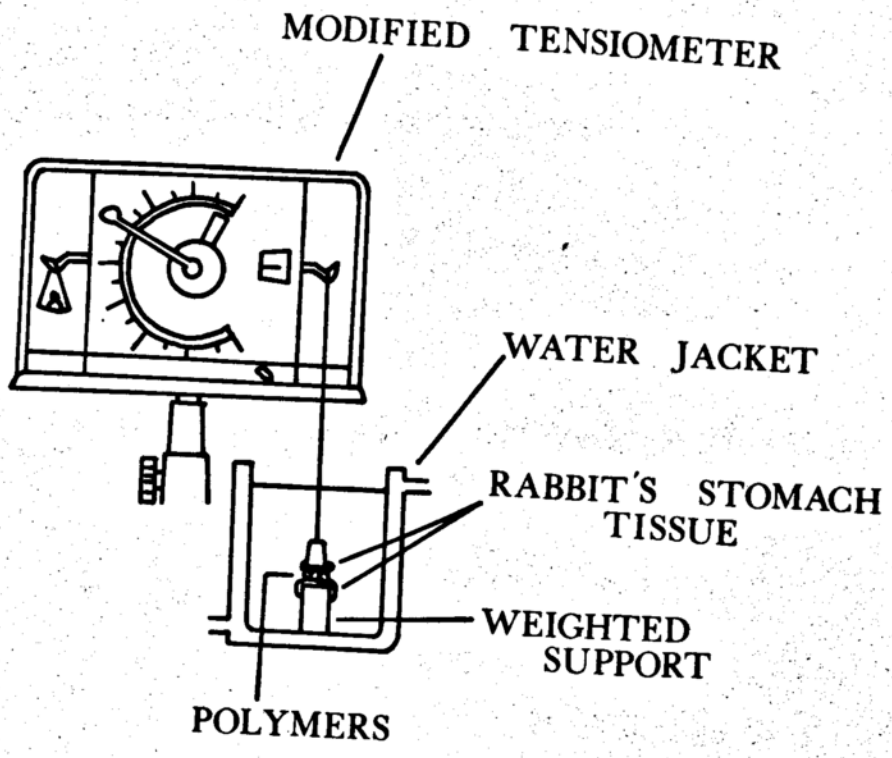
One hundred µl of assay mix was added to an assay vial. It was swirled and allowed to sit at room temperature for three minutes. One hundred µl of standard or unknown was added to the assay vial. It was vortexed for five seconds and the relative light intensity at 10 seconds was measured.

5. Tensile strength measurement

The tensile strength of mucin-mucin and polymer-mucin interactions was measured using a modified tensiometer (figure 11[6]). A rabbit stomach tissue was mounted onto a weighted support and placed in a pH 2 isotonic phosphate buffer solution. Another piece of stomach (with muscle removed) was mounted onto a stopper of known weight. Copolymer particles of 30/40 mesh cut were hydrated in pH 2 isotonic

Figure 11: Modified tensiometer

(From reference 6)

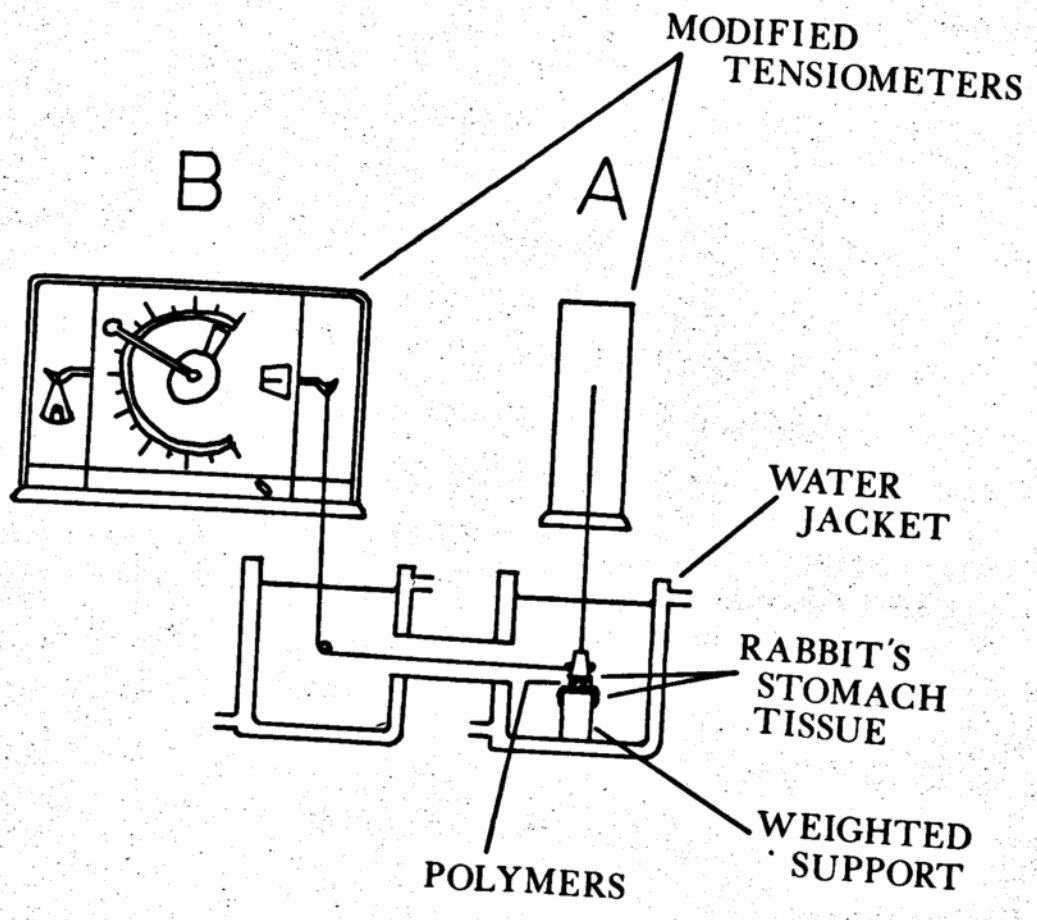

MODIFIED TENSIOMETER
WATER JACKET
RABBIT'S STOMACH TISSUE
WEIGHTED SUPPORT
POLYMERS

phosphate buffer for 24 hours. The fully hydrated copolymer particles were placed on top of the mucin surface of the tissue on the stopper. The weight of the stopper, tissue and copolymer was balanced by using a counter weight on the left hand side. The polymer surface or mucin surface (without polymer particles) on the upper stopper was allowed to come in contact with the mucin surface on the lower weighted support. The full weight of the stopper was applied to the interfacial region for one minute (or other specified period of time). The mucin-mucin or polymer-mucin interactions was measured by increasing the weight on the left hand side until a separation at the interface was observed.

6. Shear strength measurement

There are two kinds of strengths, tensile and shear, involved in mucoadhesion. A dual tensiometer apparatus was used to measure the shear strength in mucoadhesion (figure 12).[185] The basic set-up for tensiometer "A" is the same as that in figure 11. Tensiometer "A" was used to counterbalance the known weight of stopper. Tensiometer "B" was used to measure the shear strength of mucin-mucin interaction at different stopper's weight. The shear strength measurements were done in pH 2 and pH 4 isotonic phosphate buffers at 37°C.

Figure 12: Dual tensiometer


MODIFIED
TENSIOMETERS
B
A
WATER
JACKET
RABBIT'S
STOMACH
TISSUE
WEIGHTED
SUPPORT
POLYMERS

7. Effect of expanded nature of mucin network to adhesion

The contribution of the expanded nature of the mucus network was studied using: 1) a multivalent ion, calcium, which is known to precipitate mucin;[156-158] and 2) a chelating agent, EDTA, that binds multivalent ions. pH 2 isotonic phosphate buffer was used when studying the effect of calcium to mucoadhesion. The tonicity of the buffer was adjusted by using calcium chloride. The results were then compared with those that used sodium chloride to adjust tonicity. pH 4 isotonic phosphate buffer was used when EDTA was used to adjust the tonicity of the buffer. The results were then compared with those that used sodium chloride to adjust tonicity.

8. Effect of expanded nature of the polymer network to adhesion

a) Hydration study of cross-linked copolymers using buffer of different ionic strengths.

For a charged macromolecule in solution, the fixed charges established a swelling force, or the swelling pressure, or a net osmotic pressure across the membrane.[144] This different in osmotic pressure will be the driving force for solvent to enter the gel from the more dilute external bulk solution. Thus, degree of hydration of the polymer network is

influenced by the ionic strength of the external bulk solution.

The hydration of the series of synthesized copolymer (x-AA/MM) was studied at different ionic strengths using the same procedure as in D3.

b. Tensile strength of cross-linked copolymers at different degree of hydration

The degree of hydration was controlled by using pH 2 phosphate buffer of different ionic strengths. The tensile strength was measured by the modified tensiometer (figure 11).

c. Hydration study of polycarbophil with buffers of different calcium concentrations

pH2 phosphate buffer was prepared and placed in different measuring cylinders. Different amounts of calcium chloride were added. Five hundred mg of polycarbophil was added to each cylinder slowly with stirring. The flasks were left undisturbed for 24 hours to allow the suspension to settle. The volume of the sediment was then recorded.

d. Tensile strength of polycarbophil with two different percentages of calcium by weight

Carbopol® Ex-83 and EX-140 with 20% and 13% calcium by weight respectively were gifts from the BF Goodrich Company (Cleveland, OH). Carbopol® EX-83 and EX-140 were hydrated in pH2 isotonic phosphate buffer solution for 24 hours. Tensile strength measurements were done by the

modified tensiometer (figure 11).

9. Time dependency of mucoadhesion

The penetration rate between macromolecules depends on the diffusion coefficients of both interacting macromolecules. The diffusion coefficient was found to depend on molecular weight and decreased significantly as the cross-linking density increased.[186] Segmental mobility of the polymer molecule generally governs the strength of adhesion.[130] Thus, the flexibility of the bioadhesive polymer and mucus glycoprotein molecules are important parameters in mucoadhesion. The exact interpenetrating depth required to achieve adequate mucoadhesion is not known, but the representative mean diffusional path, s, for macromolecules is estimated as in equation (3),[125] $s = (2tD)^{1/2}$. If interpenetration process is important, and estimation of the mean diffusional path is correct, then the depth of the mean diffusional path should be a time dependent process. In other word, the mucoadhesion should be a time dependent process.

Thus, tensile strengths of mucin-mucin interaction and mucin-polymer interaction were determined using the modified tensiometer (figure 11). The contacting time between the two interacting surfaces was varied, and the corresponding tensile strength required for separation determined. The experiment was done at 37°C using pH2 isotonic phosphate buffer.

10. Temperature dependency of mucoadhesion

In equation (6), $D = D_o \exp(-E/RT)$, the experimental diffusion coefficient, D, was shown to have an exponential temperature dependence of the Arrhenius type, where E is the experimental activation energy for diffusion or for mobility of the segment chains. In order to check if the interdiffusional process is temperature dependent, tensile strength of the polymer-mucin interaction was determined at different temperatures. The apparatus used was the modified tensiometer (figure 11), and the experiment was done using pH2 isotonic phosphate buffer.

11. Determination of average mesh size of copolymers

The average mesh size, $\overline{\xi}$, of the network structure can be calculated by using equation (7),[149] $\overline{\xi} = \alpha_s (2nl^2)^{1/2}$, where n is the number of links (C-C bonds) between two cross-links, l is the bond length (1.54 Å), and the linear deformation coefficient, α_s, can be approximated by equation (8), $\alpha_s = (V/V_o)^{1/3}$. Volume of the swollen polymer was obtained from hydration studies, and volume of the unswollen polymer was obtained by measuring the volume of cyclohexane displaced by known weight of copolymer.

IV. RESULTS AND DISCUSSION

A. Synthesis and quantitative characterization of copolymer

1. Percent yield of copolymers

The percentage yield of the synthesized copolymers is shown in table 2.[185] The percentage yield was low due to the long washing process. Such a long washing procedure was needed to make sure that all the noncross-linked polymer chains, and unreacted monomer and reagents were removed.

2. Quantitative characterization of copolymers

a. Acridine orange binding technique

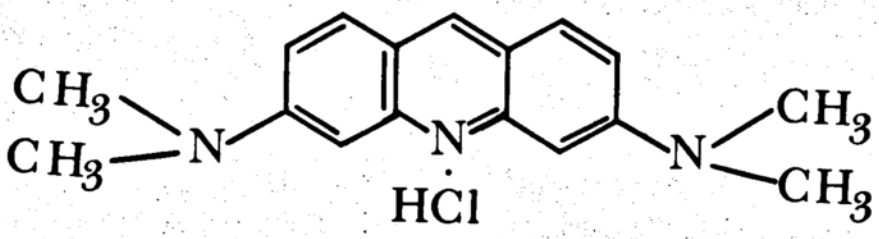
The chemical structure of acridine orange is shown in figure 13. Adsorptivity of acridine orange onto the polystyrene centrifuge tube was determined to be negligible during the course of the experiment.[183] From previous studies,[183] it was proposed that when acridine orange binds to polycarbophil, one acridine orange molecule binds to approximately 4 charge sites. It was found that, the charge density of polycarbophil (cross-linked polyacrylic acid) can be experimentally estimated in term of equivalent mass by using the acridine orange binding technique.[183]

Thus, the same technique was used to determine the equivalent masses of the series of synthesized copolymers. Once the equivalent mass was determined then the percent composition of acrylic acid and methyl methacrylate will be back-calculated.

TABLE 2 : PERCENTAGE YIELD OF SYNTHESIZED COPOLYMERS OF DIFFERENT PERCENT COMPOSITIONS OF ACRYLIC ACID

ACRYLIC ACID / METHYL METHACRYLATE RATIO	PERCENT YIELD
10/0	58.5
9.5/0.5	56.0
9.0/1.0	58.8
8.5/1.5	46.5
8.0/2.0	47.3
7.0/3.0	58.2
6.0/4.0	72.1
5.0/5.0	79.0
0/10	58.1

Figure 13: Chemical structure of acridine orange



	pKa
Heterocyclic nitrogen	10·10
Dimethylamino nitrogen	-3·15

But the experimental equivalent mass was found to follow an irregular pattern as the ratio of acrylic acid:methyl methacrylate increased in the original reaction mixture. Thus, percent composition of the synthesized copolymers cannot be determined by using the acridine orange binding technique, although it can be used to determine the equivalent mass of polycarbophil. One possible explanation for this is that for polymer with one monomer, e.g., polycarbophil, the distribution of the charged group, e.g., carboxyl group, is uniform, thus each acridine orange molecule covered a constant number of charged groups, and equivalent mass can be determined. For copolymer with monomeric units randomly distributed, e.g., polyacrylic acid-methyl methacrylate, the distribution of the charged groups are not uniform, thus the number of charged sites covered by each acridine orange molecule will not be constant. As a result, the equivalent mass cannot be determined. It is concluded that the acridine orange binding technique can be used to determine the equivalent mass of polymers with only one kind of monomer.

b. Infrared spectrophotometry

Standard curves for cross-linked polyacrylic acid and cross-linked polymethyl methacrylate are shown in figure 14 and figure 15 respectively. The experimental percent compositions of acrylic acid and methyl methacrylate in different cross-linked copolymers are shown in table 3, and are

Figure 14: Standard curve of infrared absorption of cross-linked polyacrylic acid

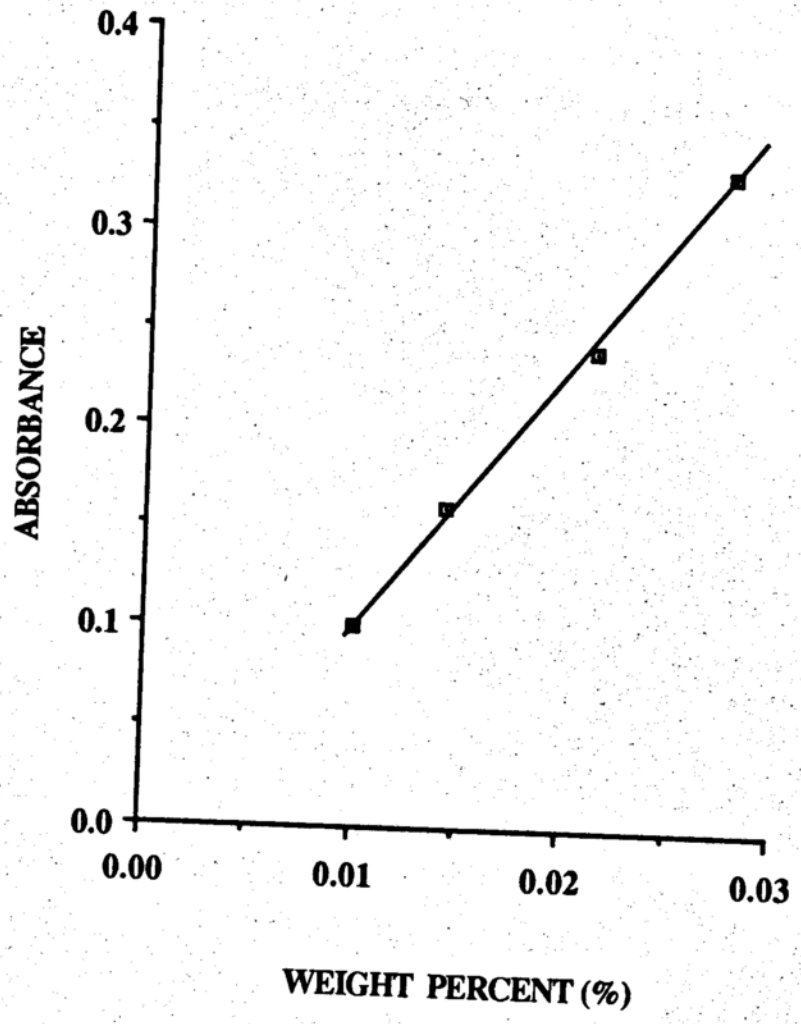
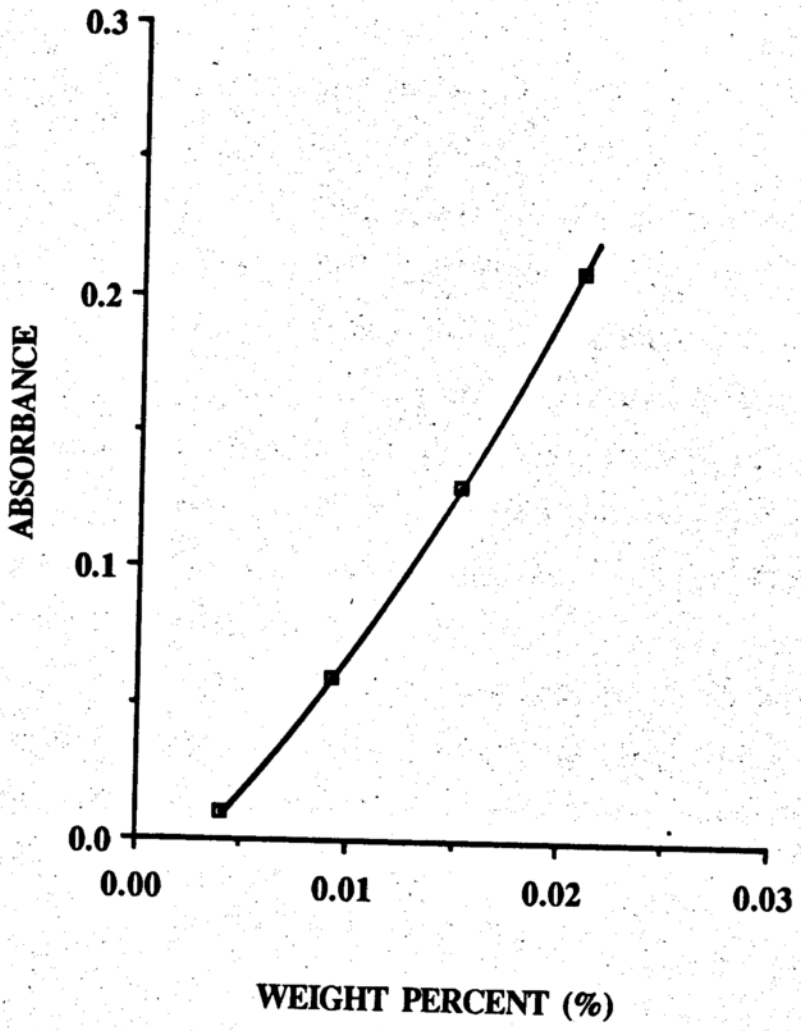

0.4
0.3
0.2
0.1
0.0
ABSORBANCE
0.00
0.01
0.02
0.03
WEIGHT PERCENT (%)

Figure 15: Standard curve of infrared absorption of cross-linked polymethyl methacrylate


0.3
0.2
0.1
0.0
ABSORBANCE
0.00
0.01
0.02
0.03
WEIGHT PERCENT (%)

found to be in good agreement with starting fractions of the component parts.

B. Viability of rabbit stomach tissue

1. Two-dye method

A two-dye method[187] (neutral red and trypan blue) was used to distinguish vital from nonvital cells. Neutral red reacts with vacuoles or granules that contain a phospholipoprotein complex, acid phosphatase, lipase, and some alkaline phosphatase, whereas trypan blue stains dead cells but not live cells. Thus, by using the two-dye method, viable cells are stained red, dead cells are stained blue and can be differentiated. If a cell contains both colors, it was damaged in processing.[187]

The two-dye method was used in the rabbit stomach tissue. For dead tissue, the cells were stained blue, whereas, for tissue that was kept in the aerated, chilled normal saline solution, the cells were stained red for up to 2.5 hours.

2. Bioluminescent method

a. Standard curve

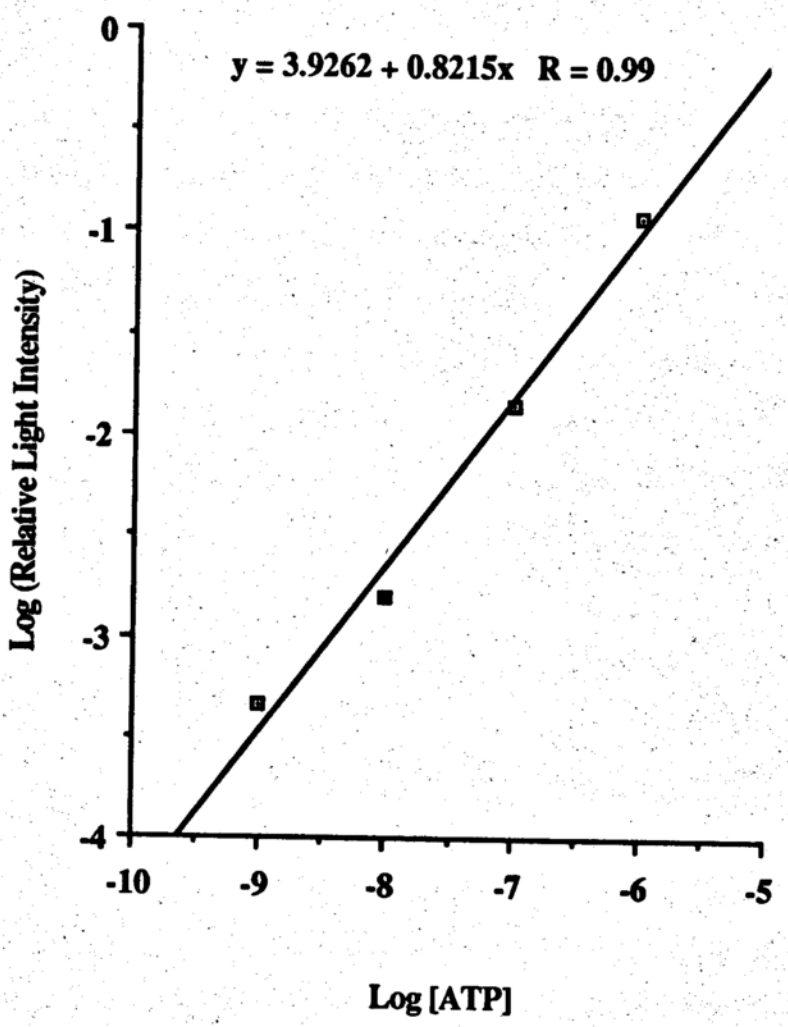
Standard solutions of concentration of 10^{-9} to 10^{-6} g/100μl were prepared via serial dilution of the ATP standard stock solution. The standard curve is shown in figure 16. A straight line with correlation coefficient of 0.99 was obtained. The standard curve was prepared on the same day as the sample and was used to calculate the ATP levels of the unknown samples.

TABLE 3: EXPERIMENTAL PERCENT COMPOSITIONS OF THE SYNTHESIZED COPOLYMERS USING AN IR SPECTROSCOPIC METHOD

Acrylic acid / methyl methacrylate	Experimentally determined percent acrylic acid	Experimentally determined percent methyl methacrylate
9.5/0.5	89.4(0.4)	10.6(0.4)
9.0/1.0	89.0(0.5)	11.0(0.5)
8.5/1.5	83.6(1.2)	16.4(1.2)
8.0/2.0	79.8(0.5)	20.2(0.8)
7.0/3.0	68.8(0.5)	31.2(0.7)
6.0/4.0	59.3(2.2)	40.7(2.2)
5.0/5.0	53.1(4.1)	46.9(4.1)

Percent acrylic acid (standard error of the mean) and percent methyl methacrylate (standard error of the mean)were determined from 4 to 6 measurements.

Figure 16: Standard curve of ATP assay


y = 3.9262 + 0.8215x R = 0.99
Log (Relative Light Intensity)
0
-1
-2
-3
-4
-10
-9
-8
-7
-6
-5
Log [ATP]

b. Samples

Changes in intracellular ATP concentrations of the stomach tissue were measured by means of bioluminescence. The ATP levels at different time points after sacrifice of animal were measured (figure 17). It was shown that there was a slight decrease in ATP concentration with time but the difference between the three values were statistically insignificant. Thus, it seems that the stomach tissue is viable for at least 2 hours in chilled and aerated normal saline solution.

In all experiments, the rabbit's stomach was kept in aerated and chilled normal saline solution and was used within 30 minutes to 1 hour after the animal was sacrified. Thus, it is concluded that the tissue was viable during the course of the experiment.

C. Effect of expanded nature of the polymer network to mucoadhesion

1. Degree of hydration of copolymers

a. In pH2 isotonic phosphate buffer

(i) x-AA/MM

The degree of hydration of the cross-linked copolymers (x-AA/MM) was studied in pH2 isotonic phosphate buffer and the results are shown in figure 18. The amount of water uptake by the copolymers represents the amount of water sorbed per gram of cross-linked copolymer when the corresponding copolymers were fully hydrated. It was found that

Figure 17: A graph of amount of ATP in stomach tissue versus time

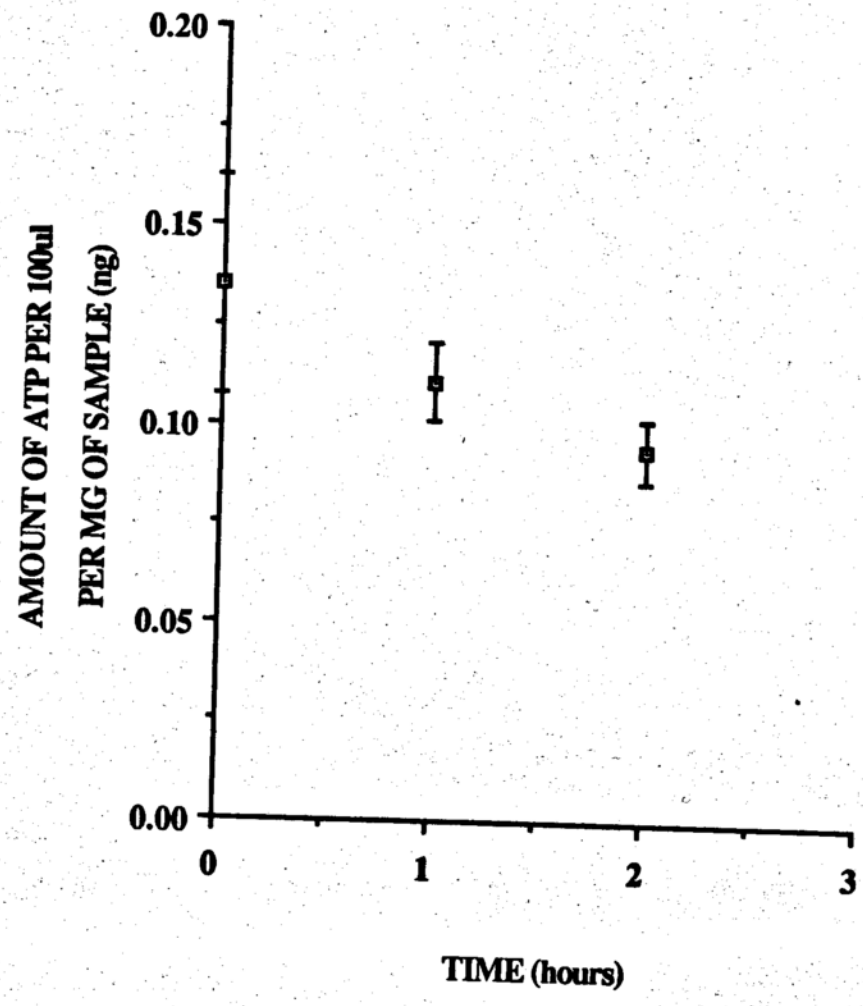
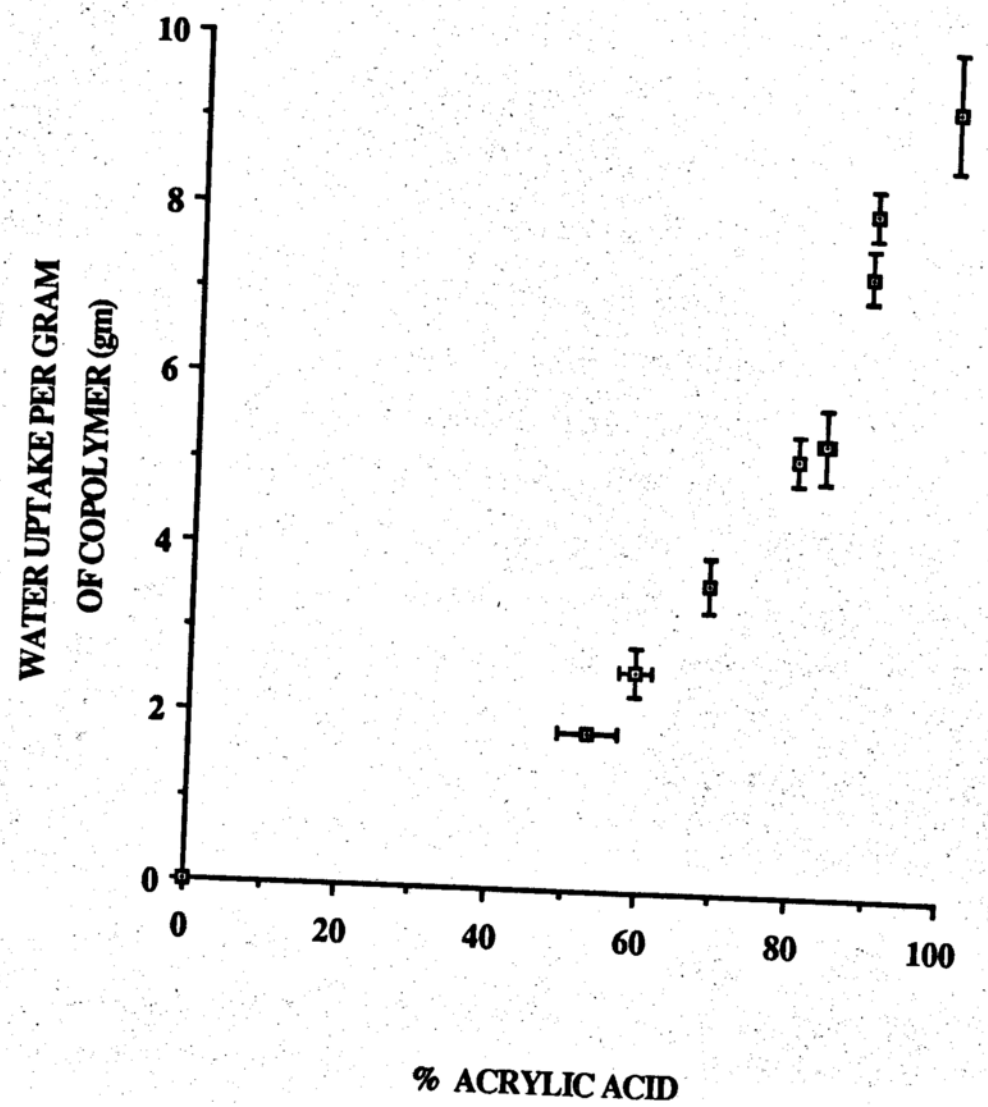

AMOUNT OF ATP PER 100ul
PER MG OF SAMPLE (ng)
0.20
0.15
0.10
0.05
0.00
0
1
2
3
TIME (hours)

Figure 18: Hydration of copolymers with different percents of acrylic acid


WATER UPTAKE PER GRAM
OF COPOLYMER (gm)
10
8
6
4
2
0
0
20
40
60
80
100
% ACRYLIC ACID

as the percent composition of acrylic acid decreased, the amount of water sorbed, i.e., the degree of hydration decreased in a similar fashion. In other word, the percent composition of acrylic acid in the copolymer influence amount of water sorbed.

(ii) Carbopol® EX-83 and EX-140

Carbopol® EX-83 and EX-140 contained 20% and 13% calcium by weight respectively. Their hydration volumes in pH2 isotonic phosphate buffer are shown in figure 19. There is a decrease in hydration volume when calcium content is increased from 0% to 13%, this may be the result of conformational change of the polymer network caused by the presence of calcium. But as the calcium content increased further up to 20%, there is an increase in hydration volume. Please refer to section-1c for possible explanation.

b. In pH2 phosphate buffers of different ionic strengths

The degree of hydration of the cross-linked copolymers (x-AA/MM) in pH2 phosphate buffer of different ionic strengths is shown in figure 20. It is found that, as the ionic strength of the media increased from $1.3x10^{-1}$M to 2.0M, the amount of water sorbed per gram of copolymer decreased. The hydration of copolymer network depends primarily on the establishment of a net osmotic pressure across the polymer network which acts as a membrane. The net osmotic pressure decreases as the ionic strength of the external bulk solution

Figure 19: Effect of calcium content on hydration volume of polycarbophil

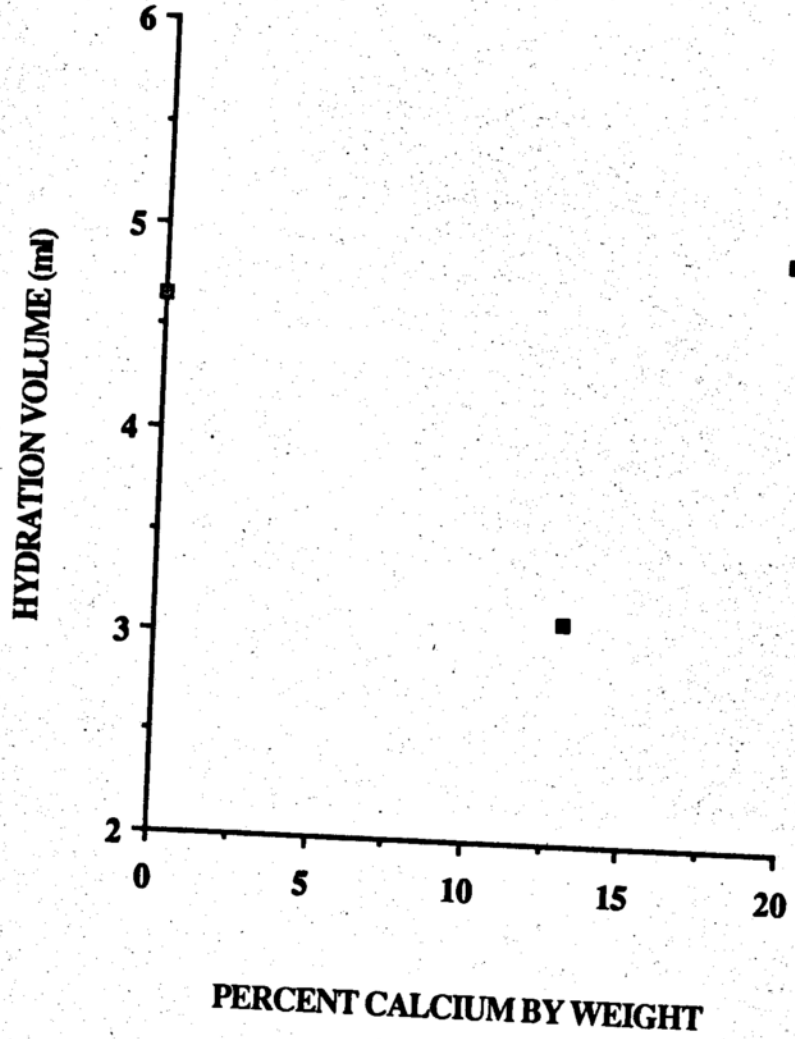
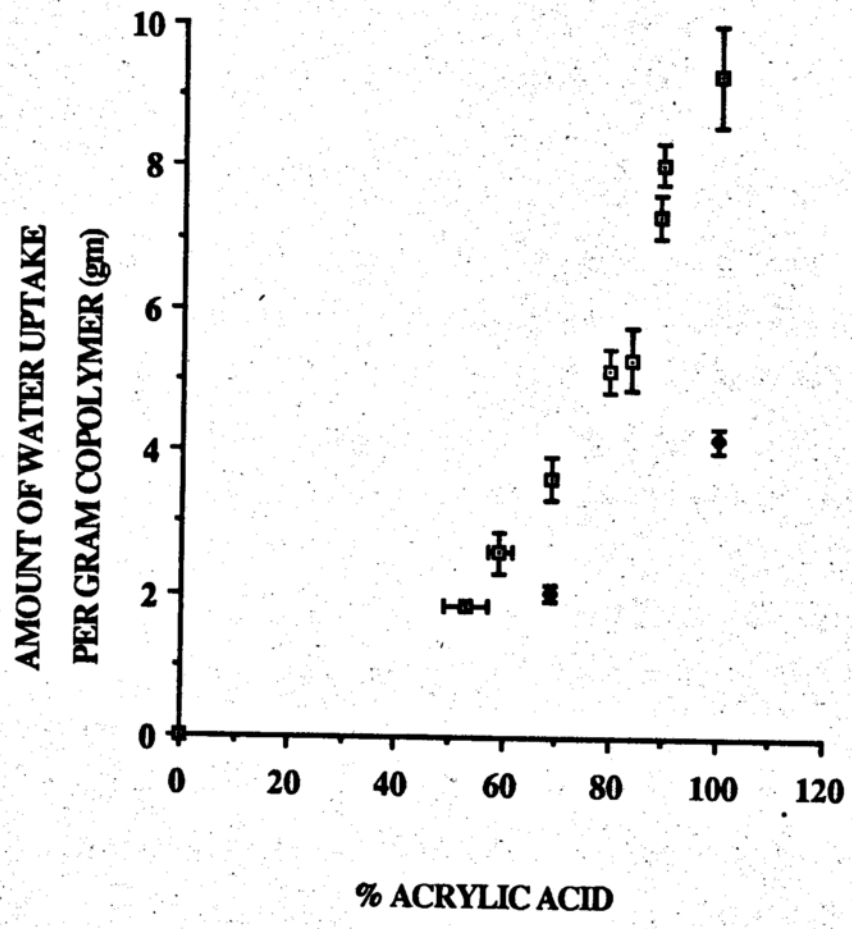

HYDRATION VOLUME (ml)
6
5
4
3
2
0
5
10
15
20
PERCENT CALCIUM BY WEIGHT

Figure 20: Amount of water uptake per gram of copolymer at different ionic strengths


AMOUNT OF WATER UPTAKE
PER GRAM COPOLYMER (gm)
10
8
6
4
2
0
0
20
40
60
80
100
120
% ACRYLIC ACID
I = 0.13 M
I = 2.00 M

increases, with corresponding decrease in degree of hydration. Thus, degree of hydration of the copolymer can be influenced by changing the ionic strength of the media.

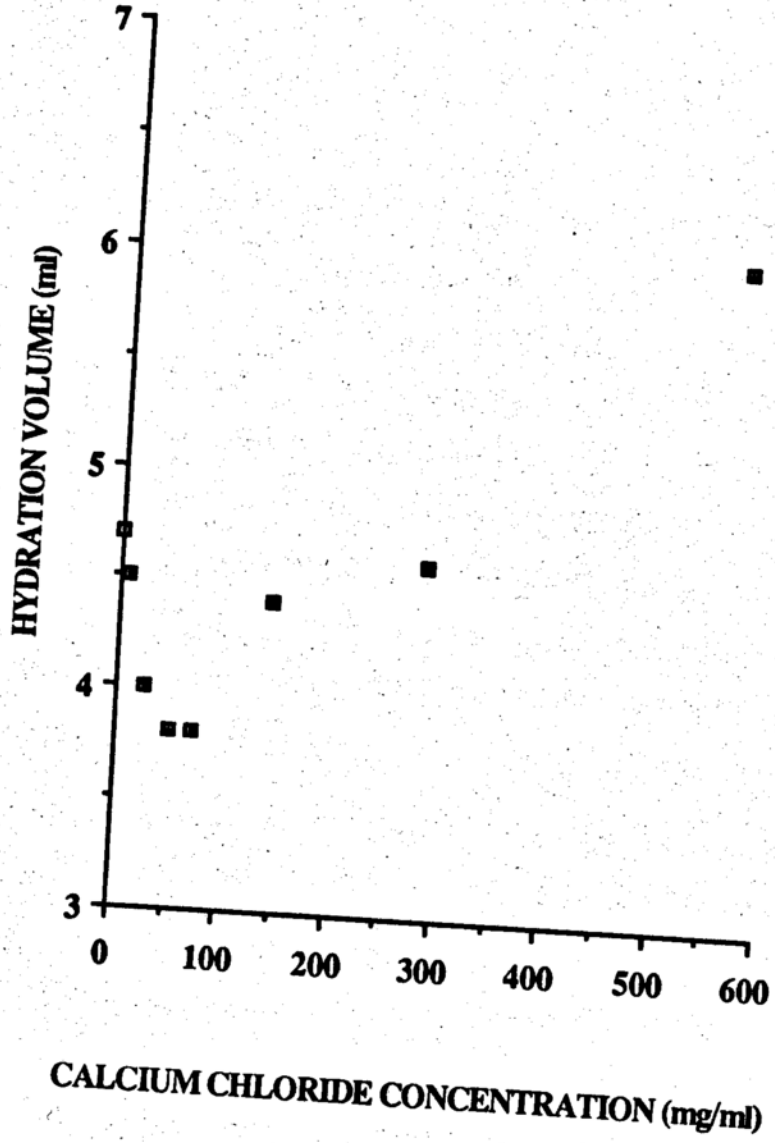
c. In pH2 phosphate buffer of different calcium concentrations

The effect of calcium on hydration volume is shown in figure 21. The hydration volume of 500mg of polycarbophil (x-AA) was found to decrease initially as the concentration of calcium increase. But at higher calcium concentration, e.g., 100mg/ml, the hydration volume of 500mg of polycarpbophil (x-AA) began to increase with increasing calcium concentration.

Calcium is known to bind to macromolecules.[156,167] Thus, the initial decrease in hydration volume might be due to a conformational change induced by the calcium ions, or by an increase in ionic strength of the medium. But at higher calcium concentration, i.e., at higher ionic strength, the hydration volume increases with calcium concentration. Calcium is known to form bridges between negative charged groups,[167] e.g., phosphate and carboxyl groups. Thus, increase in hydration volume might be the consequence of the bridging effect of calcium ions.

As the calcium concentration increased, more bridges were formed between carboxyl groups within the same or between different polymer particles, which results in a more expanded network and thus a greater hydration volume. This bridging

Figure 21: Effect of buffer's calcium concentration in hydration volume of 500mg of polycarbophil


HYDRATION VOLUME (ml)
7
6
5
4
3
0
100
200
300
400
500
600
CALCIUM CHLORIDE CONCENTRATION (mg/ml)

effect may explain the greater hydration volume of polycarbophil with 20% calcium by weight than that with only 13% calcium by weight.

2. Tensile strength of copolymers require for separation

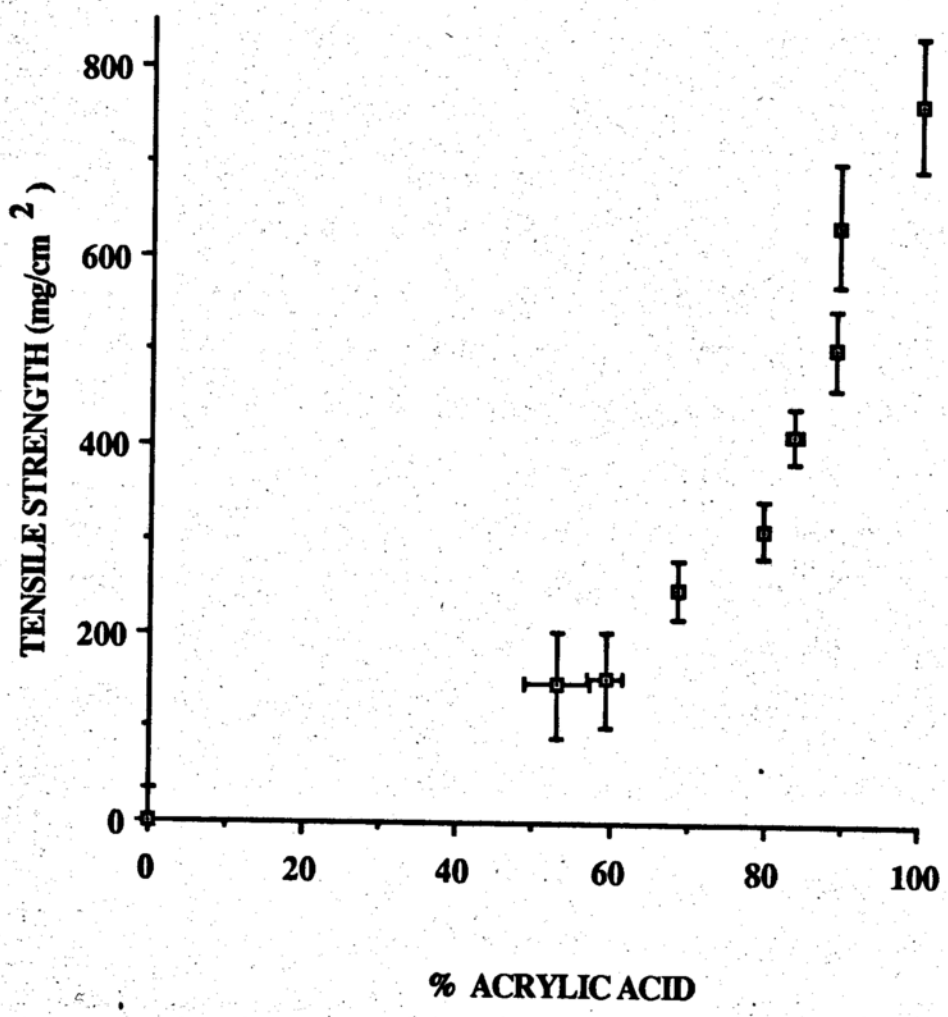
a. In pH2 isotonic phosphate buffer

The tensile strength of the synthesized copolymers (x-AA/MM) was measured. The tensile strength is related to the percent composition of acrylic acid in the copolymer (figure 22). It is found that the tensile strength of the polymer-mucin interaction decreases as the percent composition of acrylic acid decreases. Thus, the percent composition of acrylic acid directly or indirectly affects the tensile strength of the polymer-mucin interaction. The pK_a of polyacrylic acid is 4.75.[188] At pH2, the majority of the carboxyl groups of the polyacrylic acid are protonated. Thus the protonated form appears to be responsible for most of the polymer-mucin interaction.

b. In pH2 phosphate buffer of two different ionic strengths

The tensile strength of a synthesized copolymer (AA:MM=10:0) at two different ionic strengths was measured. It is found that as the ionic strength increases, the

Figure 22: Correlation of tensile strength with percent acrylic acid


TENSILE STRENGTH (mg/cm 2)
800
600
400
200
0
0
20
40
60
80
100
% ACRYLIC ACID

tensile strength decreases (figure 23).

c. With two different percents of calcium by weight

The tensile strengthes of Carbopol® EX-83 and EX-140 in pH2 isotonic phosphate buffer were measured and are shown in figure 24 and figure 25. In figure 24, it is found that as calcium content increases from 0% to 13%, the tensile strength decreases. But when the calcium content increases further to 20%, the tensile strength increases. Thus, polycarbophil with higher (20%) calcium content has greater tensile strength than that with lower (13%) calcium content. Similar result was obtained for both dry and previously hydrated polycarbophil powder (figure 25). It seems that calcium binds to the polymer network and causes some conformational changes in the network which influences the strength of mucoadhesion.

3. Correlation of degree of hydration with tensile strength (mucoadhesive strength)

As the hydration curve (figure 18) and adhesive curve (figure 22) are compared, a similar trend is observed. It appears that the percent acrylic acid in the copolymer affects hydration and adhesion in the same direction. Thus, hydration and tensile strength can be correlated, as shown in figure 26. The water uptake of 0.2% cross-linked copolymers was found to correlate very well with tensile strength, with a correlation

Figure 23: Tensile strength of AA/MM = 10/0
at two different ionic strengths

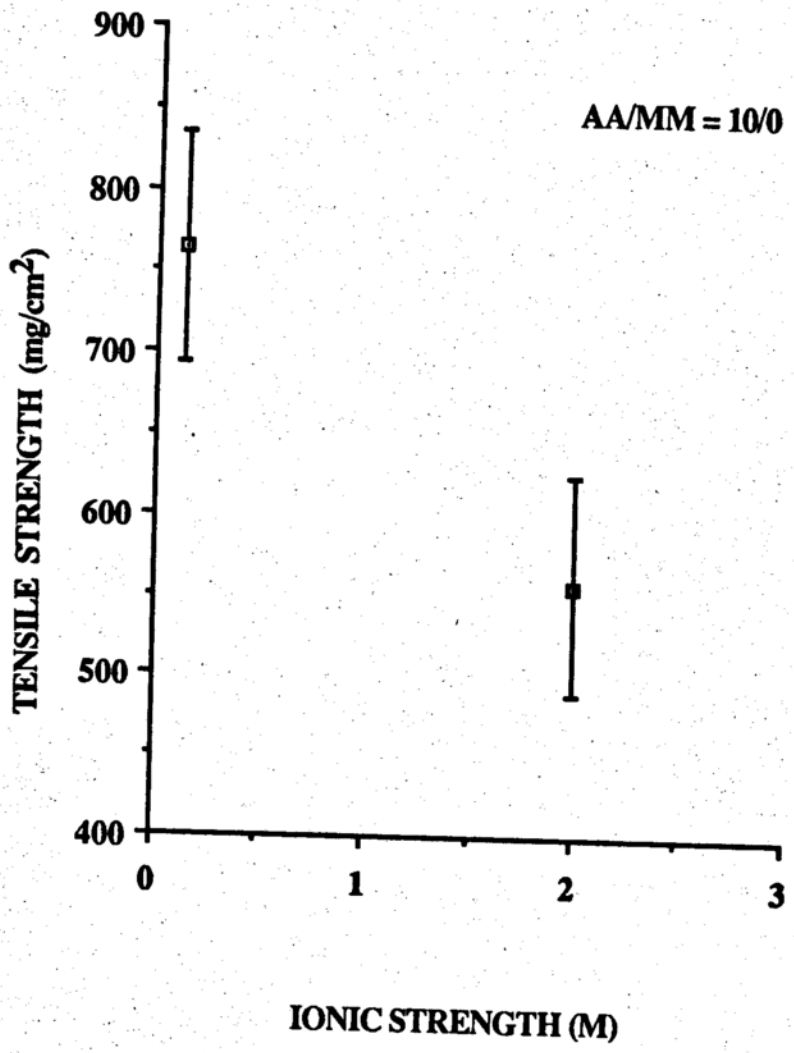

AA/MM = 10/0
TENSILE STRENGTH (mg/cm²)
900
800
700
600
500
400
0
1
2
3
IONIC STRENGTH (M)

Figure 24: Effect of calcium on tensile strength of hydrated polycarbophil

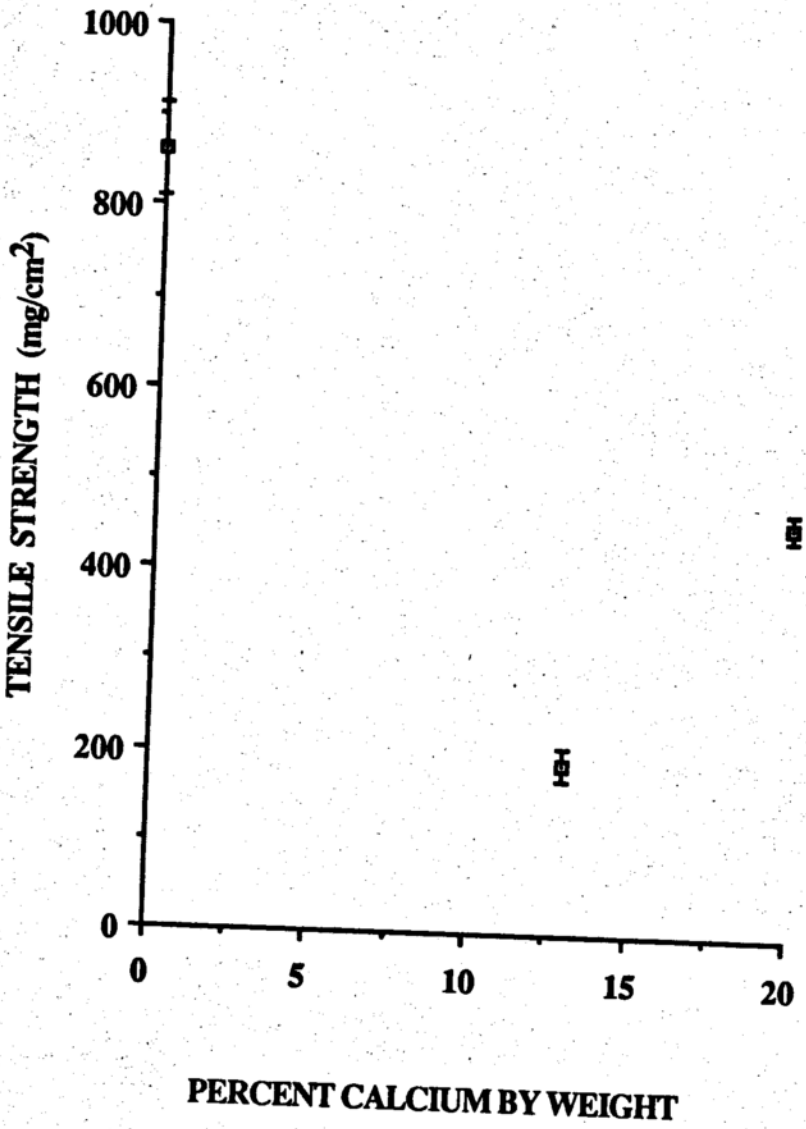

1000
800
600
400
200
0
TENSILE STRENGTH (mg/cm²)
0
5
10
15
20
PERCENT CALCIUM BY WEIGHT

Figure 25: Effect of calcium on tensile strength of dry and hydrated polycarbophil

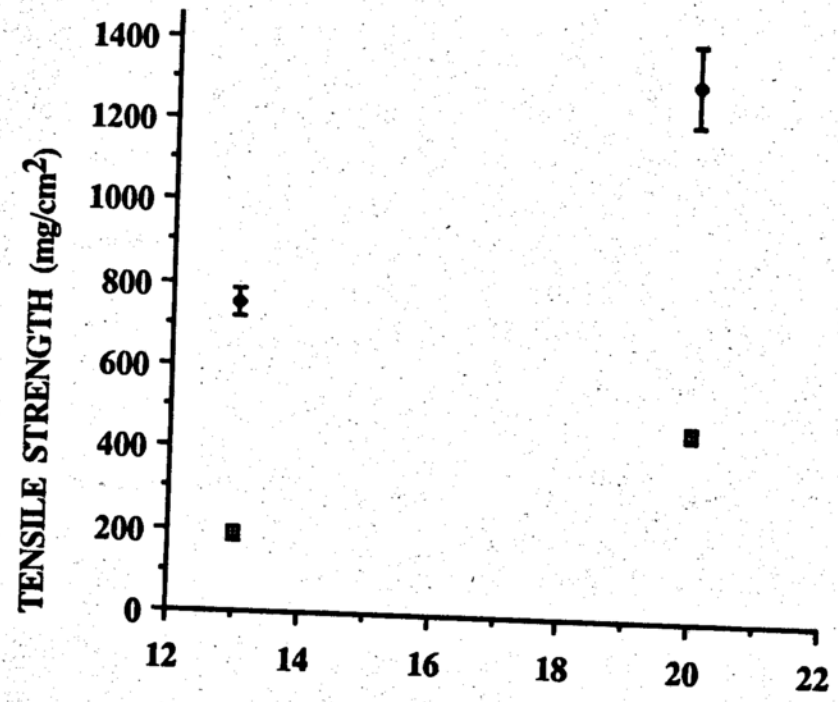
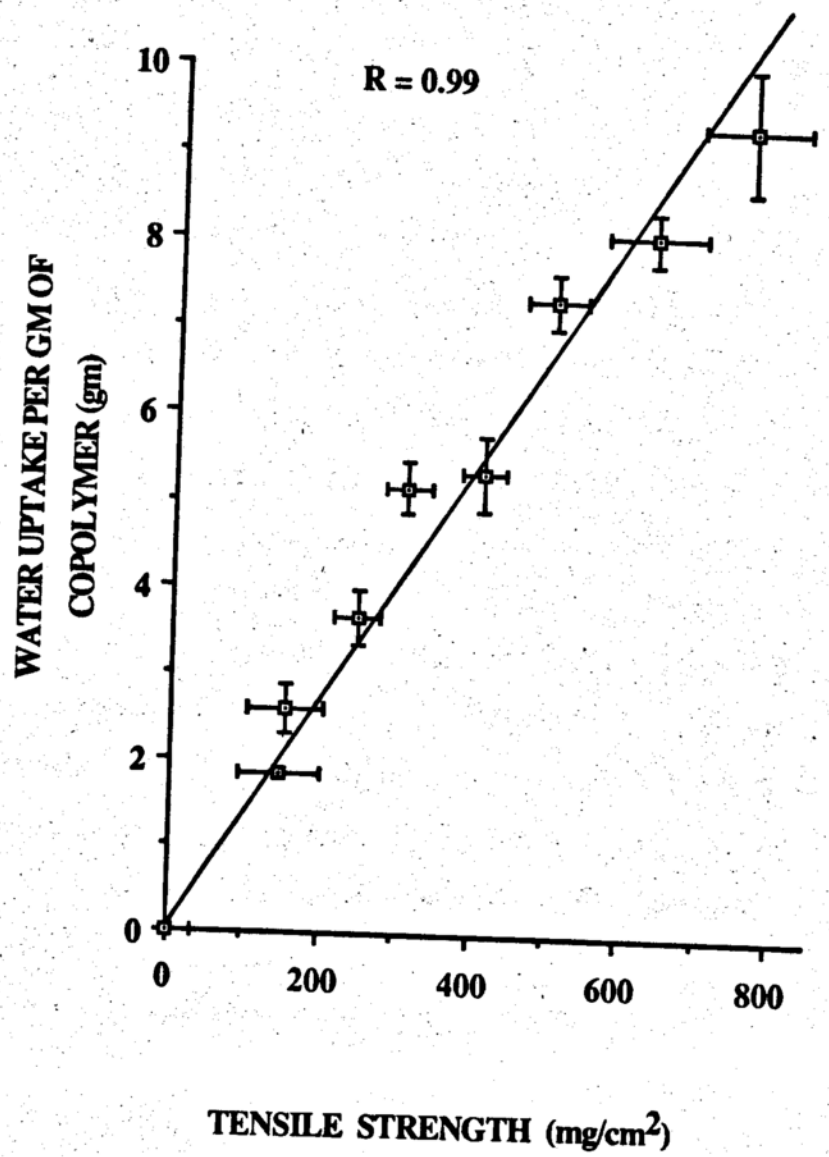

1400
1200
1000
800
600
400
200
0
TENSILE STRENGTH (mg/cm2)
12
14
16
18
20
22
PERCENT CALCIUM BY WEIGHT
Hydrated Polycarbophil
Dry Polycarbophil

Figure 26: Correlation of tensile strength with water uptake

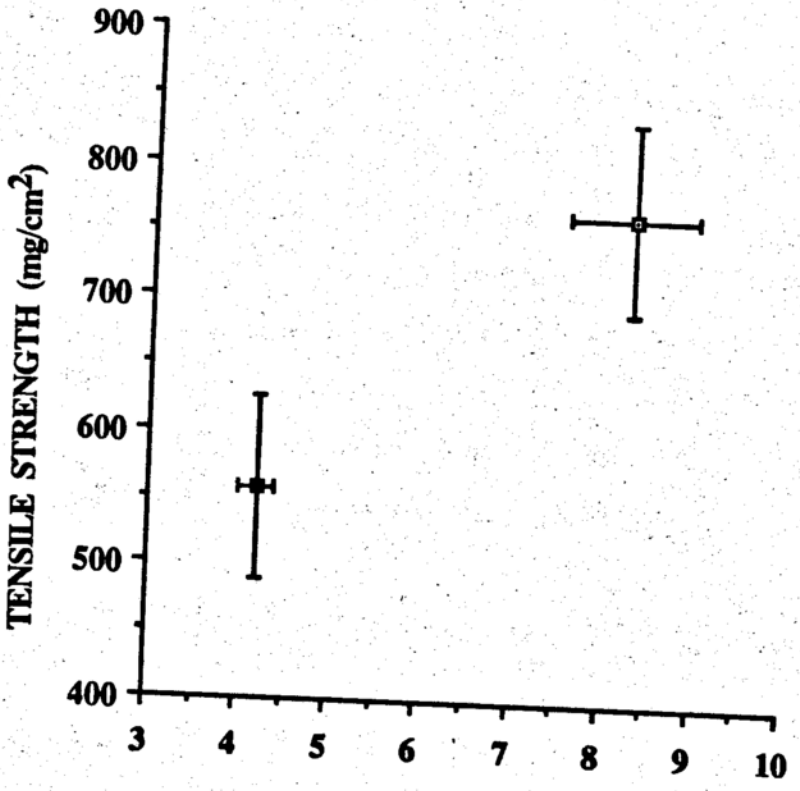

R = 0.99
WATER UPTAKE PER GM OF COPOLYMER (gm)
10
8
6
4
2
0
0
200
400
600
800
TENSILE STRENGTH (mg/cm2)

coefficient of 0.99. It appears that the expanded nature of the polymer network is very important for mucoadhesion.

When the number of available charged groups increases, the swelling pressure across the polymer increases,which results in an increase in the amount of water uptake and an increase in the degree of openness of the polymer network, since the diffusion coefficient of the solute increases with increase in mesh size and degree of swelling of the network.[147] A decrease in density of the polymer chains will increase chain segment mobility, thus enhancing the interdiffusion process, and increasing the penetration of mucin into the polymer network. As proposed by Smart et al., interpenetration and entanglement of the bioadhesive polymers to the substrate are partly responsible for their bioadhesive properties.[146] Thus an increase in interdiffusion results in an increase in physical entanglement of mucin within the polymer network, as well as a greater opportunity for hydrogen bonding, with subsequent increase in mucoadhesive strength.

The influence of the expanded nature of polymer network to tensile strength is further shown in figure 27. The degree of hydration of the polymer was controlled by adjusting the ionic strength of the hydration media. When ionic strength increases from $1.3x10^{-1}$M to 2.0M, amount of water uptake per gram of polymer decrease. At higher ionic strength, the openness of the polymer network is smaller. The decrease in the expanded nature of the polymer network decreases the mobility of the polymer and

Figure 27: Effect of ionic strength on degree of hydration and tensile strength


900
800
700
600
500
400
TENSILE STRENGTH (mg/cm²)
3
4
5
6
7
8
9
10
AMOUNT OF WATER UPTAKE PER GRAM OF POLYMER (gm)
I = 0.13 M
I = 2.00 M

mucin chains, and decreases the interdiffusion process and extent of entanglement. As a result, tensile strength decreases with increase in ionic strength.

The importance of the expanded nature of the polymer network in mucoadhesion is further supported by comparing figure 19 and figure 24. When the calcium content of the polymer increases to 13%, the hydration volume decreases, but as the calcium content increases further to 20%, the hydration volume increases. The effect of calcium on tensile strength follows a similar trend. Thus, the expanded nature of the polymer network is an important parameter in mucoadhesion.

D. Effect of applied pressure to mucoadhesion

The first stage of the bioadhesion process is the establishment of intimate contact. In this stage, the establishment of contact by viscoelastic deformation of the interacting adhesive and substrate network can be improved by increasing the pressure applied. The effect of applied pressure in mucin-mucin tensile strength and shear strength in mucoadhesion is shown in figure 28[1] and figure 29 respectively. In both tensile strength and shear strength tests, the interaction or mucoadhesive strength increased with applied force until a plateau was reached. The increase in applied pressure increases the contact area, which increases mechanical entanglement and secondary bonds formation leading to stronger mucoadhesion. As the applied pressure is increased

Figure 28: Effect of applied weight on mucin-mucin tensile strength using pH 1.2 USP simulated gastric fluid
(From reference 1)

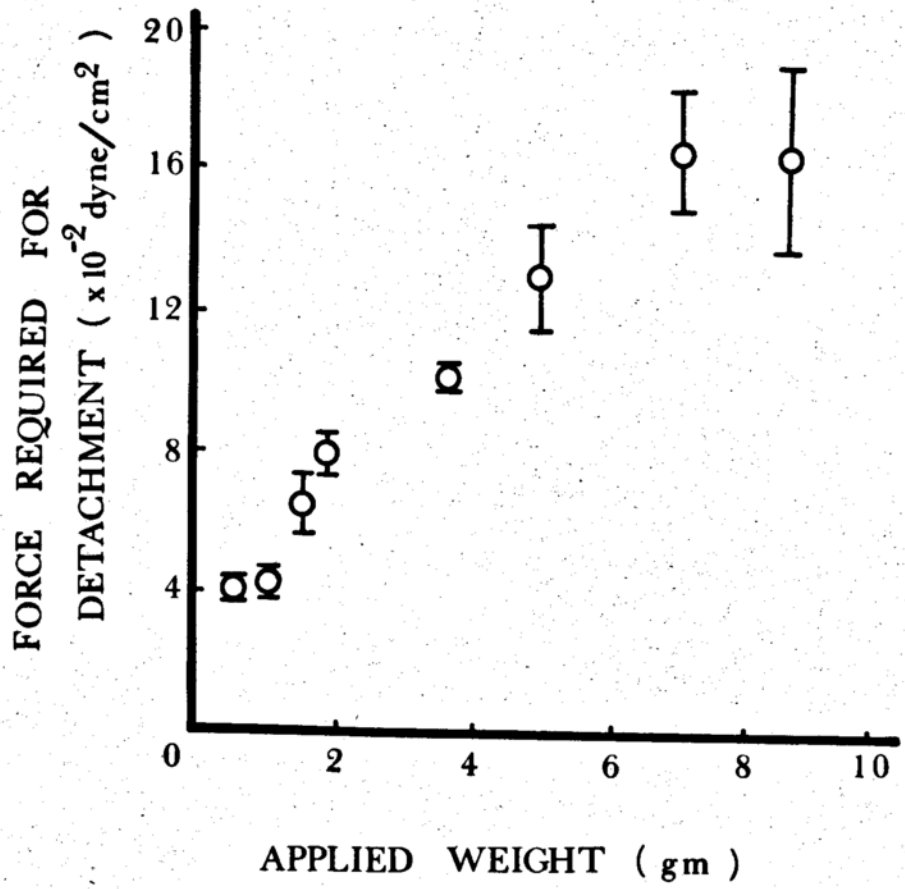
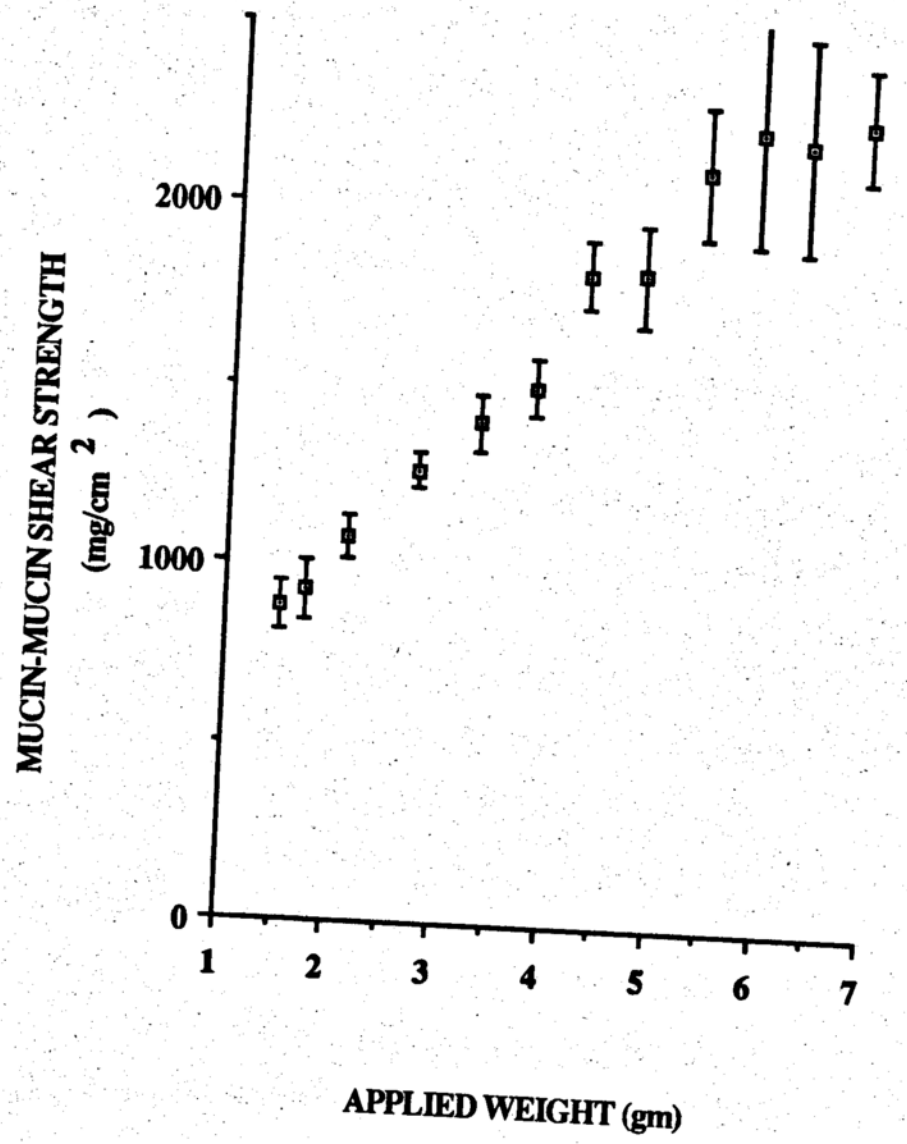

FORCE REQUIRED FOR
DETACHMENT (x 10^-2 dyne/cm^2)
20
16
12
8
4
0
2
4
6
8
10
APPLIED WEIGHT (gm)

Figure 29: Effect of applied weight on mucin-mucin shear strength using pH 2 isotonic phosphate buffer


MUCIN-MUCIN SHEAR STRENGTH
(mg/cm 2)
2000
1000
0
1
2
3
4
5
6
7
APPLIED WEIGHT (gm)

further, the molecular contact area and formation of bonds will increase in the same manner until contact area and secondary bonds formation are maximized. At this point no further increase in mucoadhesive strength is observed.

E. Effect of expanded nature of mucin network to mucoadhesion

The shear strength of the mucin-mucin interaction was studied. Results are shown in figure 29 to 32. In figure 30, the mucin-mucin shear strength in pH 2 was found to increase with applied weight and plateaued at an applied weight of 6 grams, whereas the mucin-mucin shear strength was lower, although still increasing with applied weight, when calcium chloride was used to adjust osmoticity of the phosphate buffer. As discussed earlier, calcium either directly binds to the carboxyl oxygen[167] or reduces the electrostatic charges of the anionic groups.[156] In either case, there is a change in conformation of the mucus network. The calcium-induced glycoprotein aggregation results in a reduction of the expanded nature of the mucus network. This decrease in degree of openness, or conversely increase in density of the mucus network, decreases chain segment mobility of the mucin molecule, and decreases interpenetration. Thus, a smaller physical entanglement and reduction in mucin-mucin shear strength is observed.

The mucin-mucin shear strength at pH 4 is shown in figure

Figure 30: Comparison of mucin-mucin shear strength at pH 2 in the presence of sodium chloride or calcium chloride

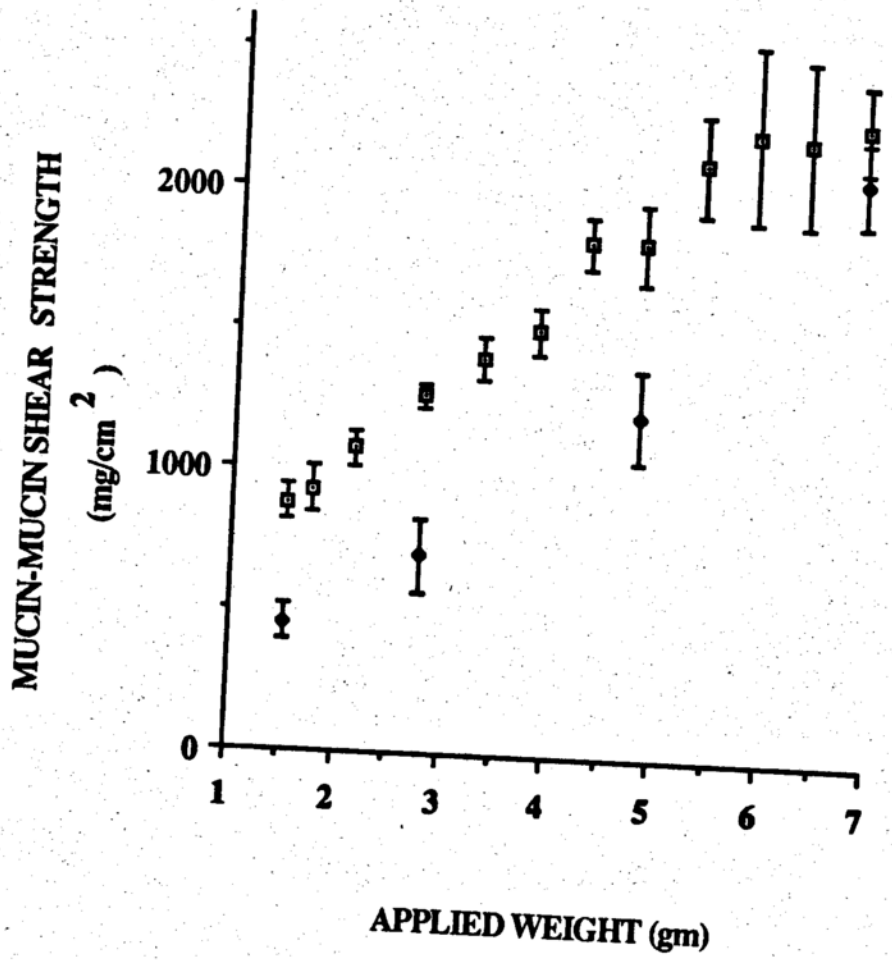

MUCIN-MUCIN SHEAR STRENGTH
(mg/cm 2)
2000
1000
0
1
2
3
4
5
6
7
APPLIED WEIGHT (gm)
Sodium Chloride
Calcium Chloride

Figure 31: Comparison of mucin-mucin shear strength pH 4 in the presence of sodium chloride, calcium chloride or EDTA

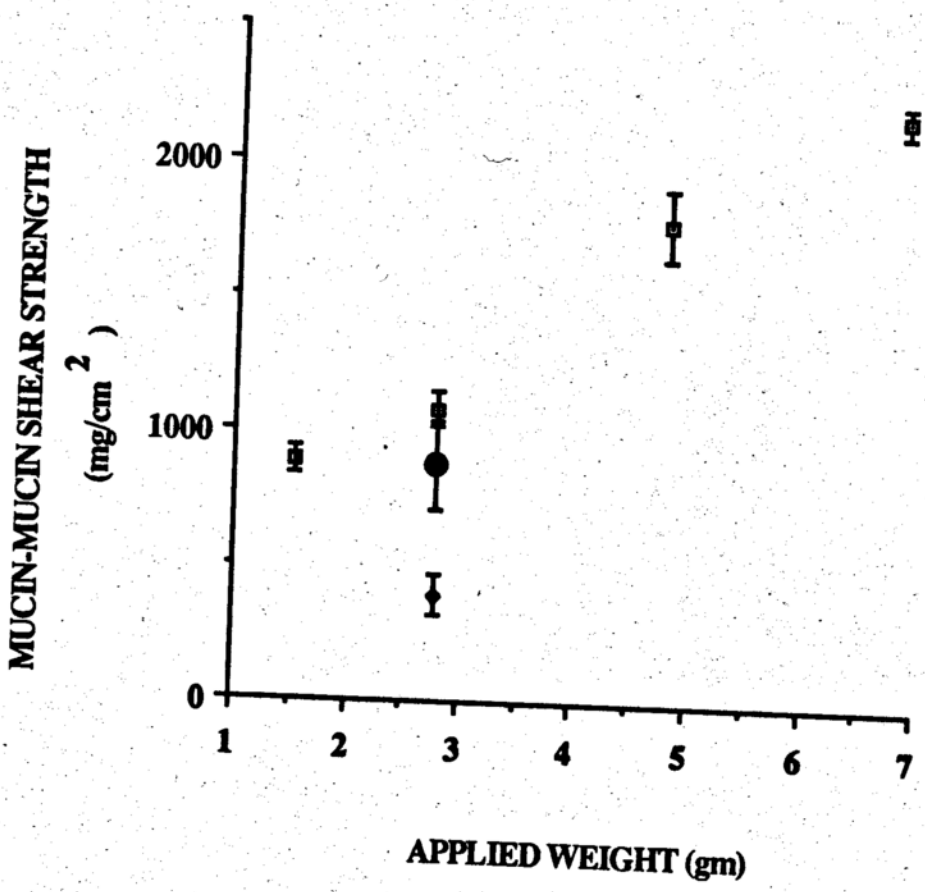
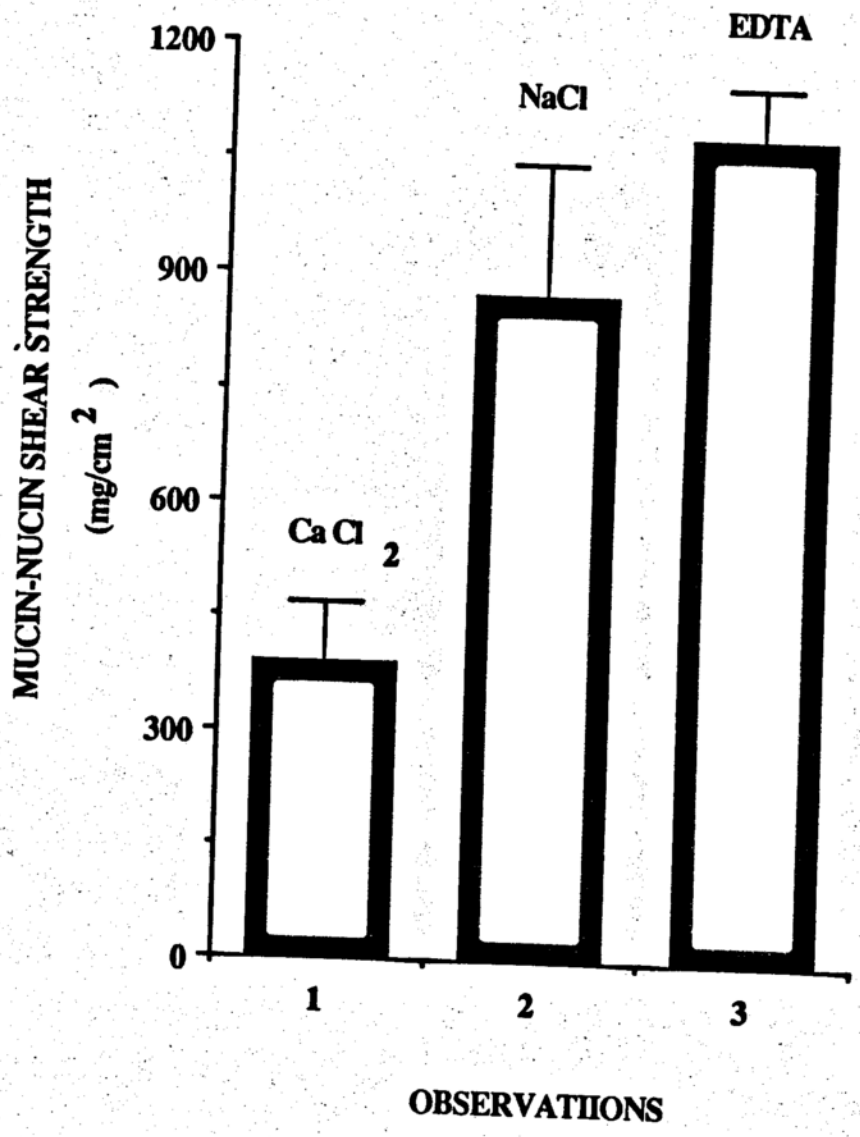

MUCIN-MUCIN SHEAR STRENGTH
(mg/cm 2)
2000
1000
0
1
2
3
4
5
6
7
APPLIED WEIGHT (gm)
E D T A
Sodium Choride
Calcium Chloride

Figure 32: Comparison of mucin-mucin shear strength at pH 4 in the presence of sodium chloride, calcium chloride or EDTA when applied weight is 2.77 grams


EDTA
NaCl
1200
900
600
300
0
MUCIN-NUCIN SHEAR STRENGTH
(mg/cm 2)
Ca Cl 2
1
2
3
OBSERVATIIONS

31, where mucin-mucin shear strength is higher and increases with applied weight when EDTA was used to adjust osmoticity of the phosphate buffer. When the effect of addition of sodium chloride, calcium chloride and EDTA are compared (figure 31, 32), the addition of EDTA was found to result in the highest mucin-mucin shear strength, followed by sodium chloride, and calcium chloride with the lowest mucin-mucin shear strength. This further supports the importance of the expanded nature of mucus in mucoadhesion, since addition of EDTA chelates calcium in the mucus layer and increases openness of the network. Thus the process of interpenetration and entanglement was enhanced. This results in an increased strength of mucoadhesion. Thus, the expanded nature of both the adhesive and substrate is an important parameter in controlling bioadhesive strength.

In addition to topological characteristics, compatability of the bioadhesives with the mucin substrate is required for good bioadhesion. Thus, structural similarities and small difference in the solubility parameters between the adhesive and substrate are desirable. In comparing the mucus network and the cross-linked polyacrylic acid, five distinct similarities are found. Both mucin and cross-linked polyacrylic acid consist of a network of macromolecules, are negatively charged, form expanded networks, hydrate greatly in an aqueous medium, and have a significant number of carboxyl groups for each cross-link between two adjacent chains, i.e., about 100 to 120 for mucin, and

790 for 0.2% cross-linked polyacrylic acid.[185]

F. Time dependency of mucoadhesion

From previous discussion, the expanded nature of both mucoadhesive and mucus influences the process of mucoadhesion. The importance of the openness of the interacting networks to mucoadhesion suggests that an interdiffusion process or interpenetration process is involved. Increase in the openness of the network will increase interpenetration between the substrate and adhesive and will result in an increase in surface area of contact and mechanical entanglement, with subsequent increase in mucoadhesive strength.

If interdiffusion is importance in mucoadhesion, then according to equation (3), $s = (2tD)^{1/2}$, the mean diffusional path, s, will depend on the diffusion coefficient and time. Thus, the mucoadhesion will be a time dependent process, i.e., the tensile strength will be a time dependent process. Figure 33 and figure 34 shown respectively that the mucin-mucin tensile strength and polymer-mucin tensile strength are both time dependent processes, i.e., tensile strength increases with increase in contacting time. In figure 35, the mucin-mucin tensile strength correlates fairly well with the square root of contacting time, with a correlation coefficient of 0.96. Thus, interpenetration is involved in mucoadhesion, and is a important process in determining mucoadhesive strength.

Figure 33: Time dependency of mucin-mucin tensile strength

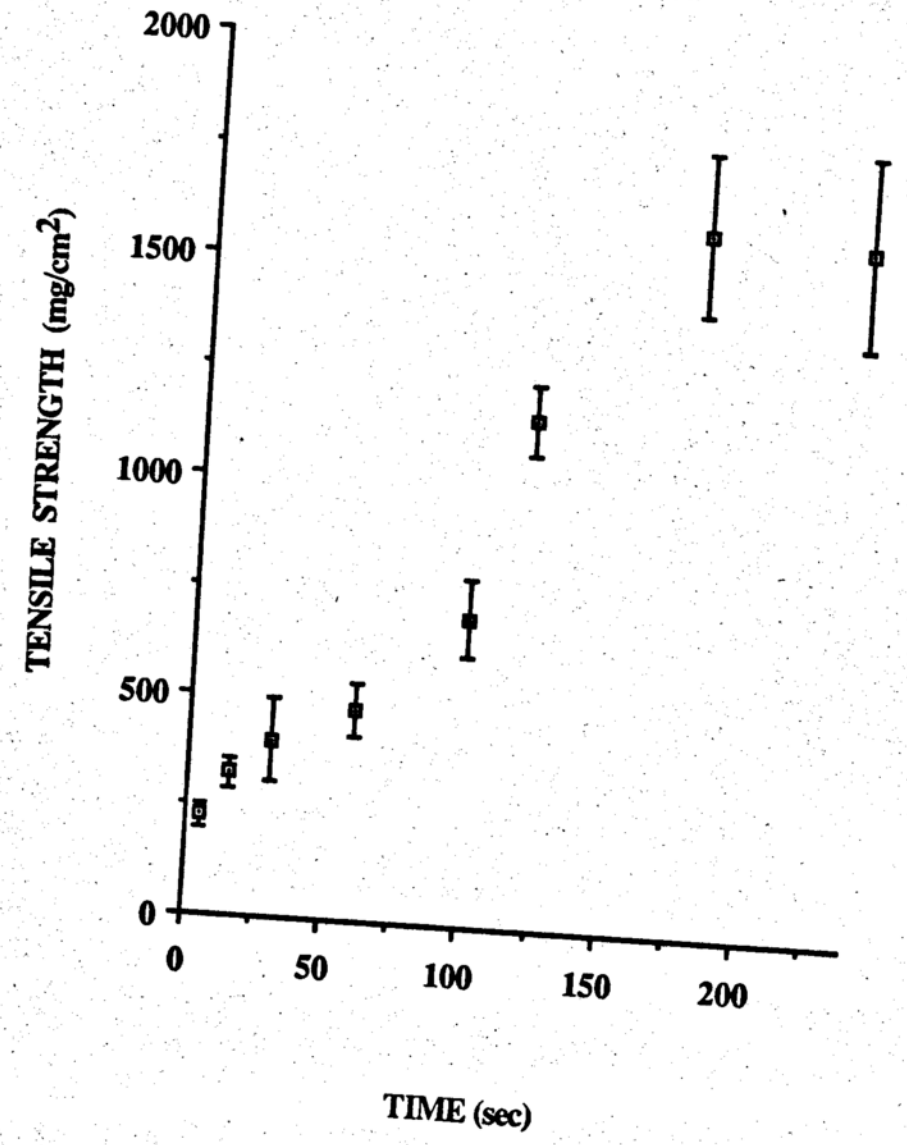

2000
1500
1000
500
0
TENSILE STRENGTH (mg/cm2)
0
50
100
150
200
TIME (sec)

Figure 34: Time dependency of polymer-mucin tensile strength

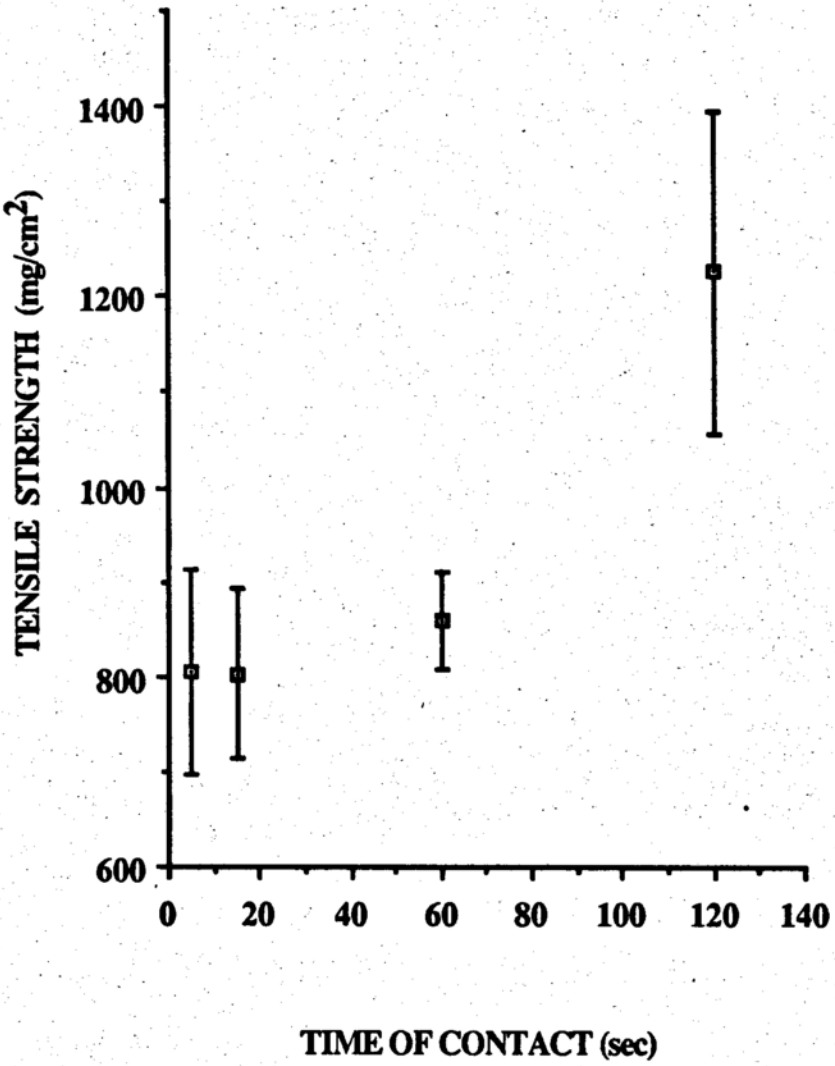
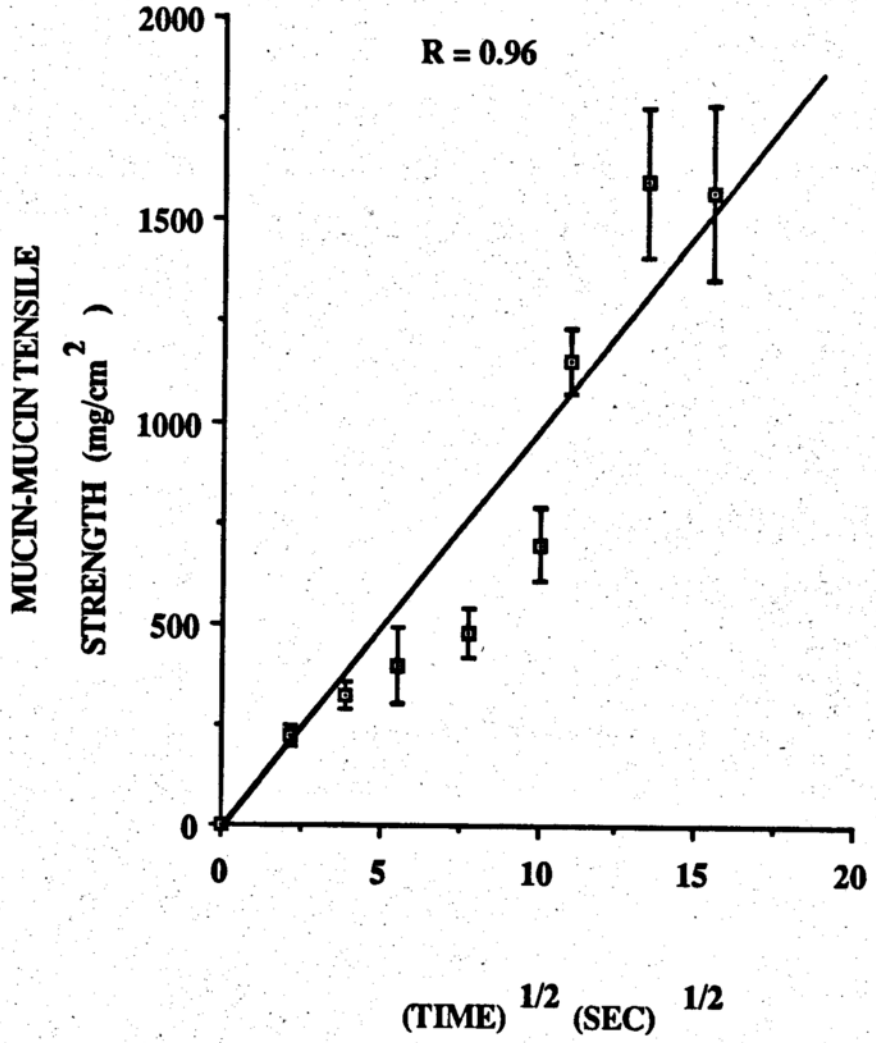

TENSILE STRENGTH (mg/cm²)
1400
1200
1000
800
600
0
20
40
60
80
100
120
140
TIME OF CONTACT (sec)

Figure 35: Correlation of mucin-mucin tensile strength with square root of time


R = 0.96
MUCIN-MUCIN TENSILE STRENGTH (mg/cm 2)
2000
1500
1000
500
0
0
5
10
15
20
(TIME) 1/2 (SEC) 1/2

In figures 33 and 35, the time dependent mucin-mucin tensile strength is shown at the earliest time points, but in figure 34, the polymer-mucin tensile strength has a time independent period of up to 60 seconds. Furthermore, the tensile strength of polymer-mucin interaction is strong at early time points. It seems that the initial contact, i.e., the first stage of mucoadhesion, is very favorable. This might be due to the favorable number of carboxyl groups on the polymer network surface, which provide a great number of charged groups for initial formation of secondary bonds.

G. Temperature dependency of mucoadhesion

In equation (6), $D = D_o \exp(-E/RT)$, the diffusion coefficient depends on temperature exponentially. From equation (3), $s = (2tD)^{1/2}$, at constant time, t,

$$s^2 = \text{constant} \times D \tag{11}$$

substitute (11) into equation (6)

$$s^2 = \text{constant} \times \exp(-E/RT) \tag{12}$$

$$2 \ln s = \text{constant} - E/RT \tag{13}$$

$$\ln s = \text{constant} - E/2R\,(1/T) \tag{14}$$

If tensile strength was assumed to relate linearly with the depth of interpenetration, then

$$\text{tensile strength} = \text{constant} \times s \tag{15}$$

then equation (14) becomes

$$\ln(\text{tensile strength}) = \text{constant} - E/2R\,(1/T) \tag{16}$$

If the assumption of linear relation between tensile strength and penetrating depth is true, then mucoadhesion will be a temperature dependent process and plot of ln (tensile strength) versus 1/T will be a straight line.

The non-Fickian temperature dependence of tensile strength at time = 2 minutes is shown in figure 36. In figure 37, the plot of ln (tensile strength) versus 1/T is shown to be a straight line with correlation coefficient of 0.99. Thus, the assumption of linear relation between tensile strength and mean diffusional depth is valid, and interpenetration/ interdiffusion is a temperature dependence process with activation energy of 30.5 kJ/mole.

H. Relationship of interdiffusion with average mesh size of copolymer network

The average mesh size of the synthesized copolymers is shown in table 4 and figure 38. The average mesh size of the polymer network is found to correlate very well with percent composition of acrylic acid with correlation coefficient of 0.99.

As the openness of the network increased, the tensile strength increased at the same time. It is intuitive that as the average mesh size of the network increased, the diffusion coefficient increased in a similar fashion. In order to understand the mechanism of mucoadhesion, it is important to find out the relationship between diffusion coefficient and average mesh size. Let's assume

Figure 36: Effect of temperature on polymer-mucin tensile strength

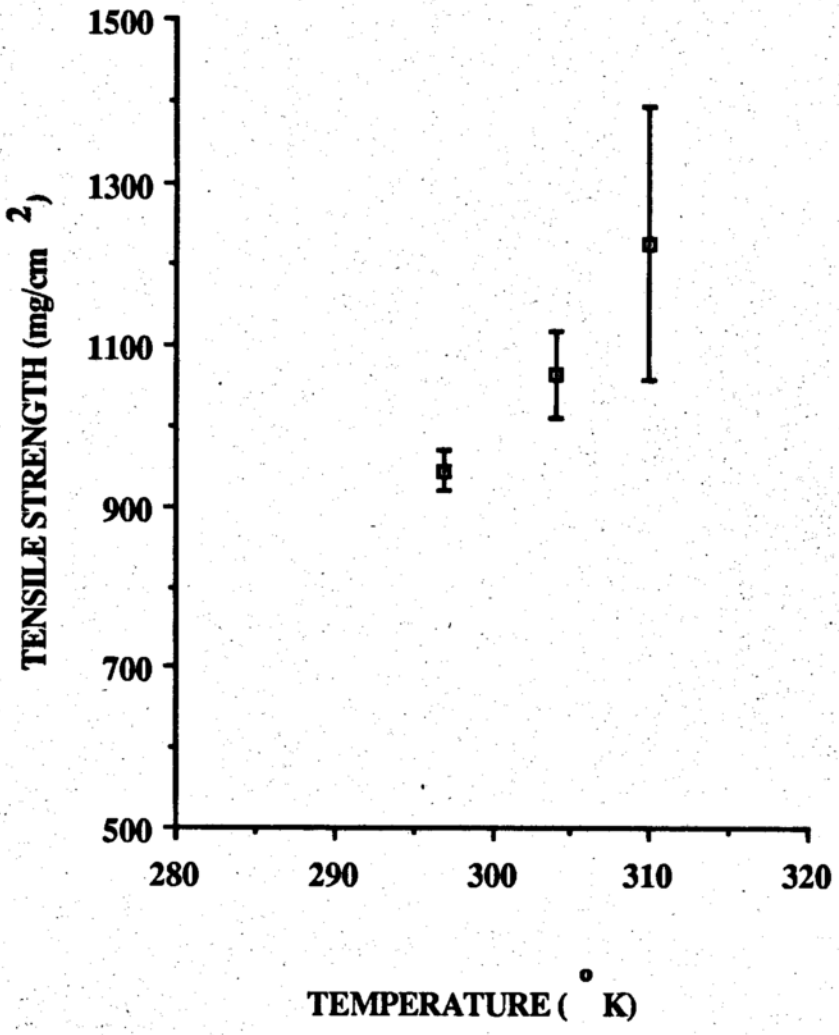

1500
1300
1100
900
700
500
280
290
300
310
320
TENSILE STRENGTH (mg/cm 2)
TEMPERATURE (° K)

Figure 37: A graph of ln (tensile strength) versus 1/T

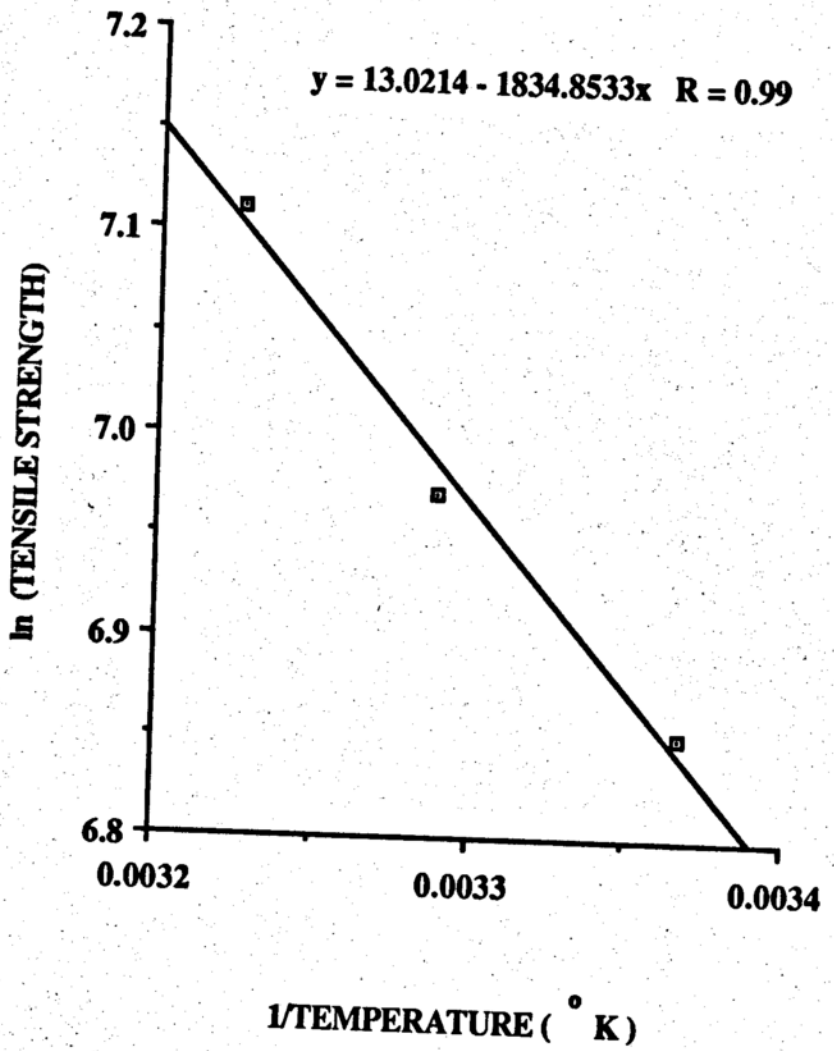
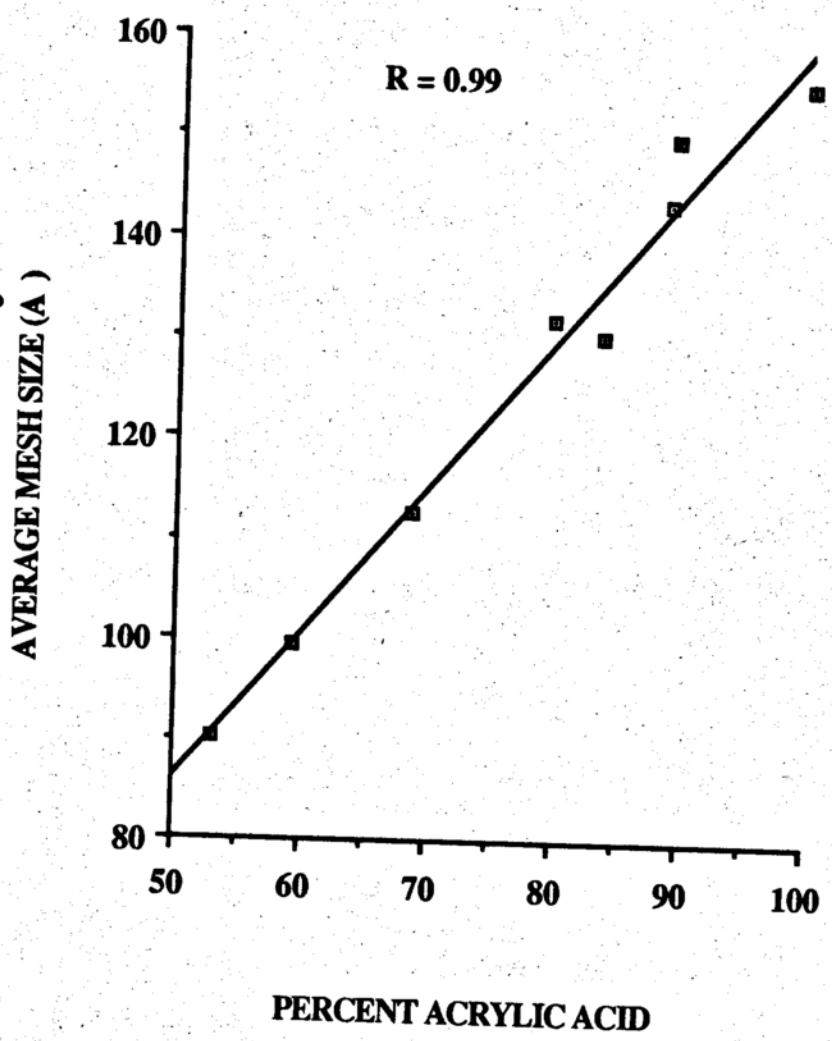

y = 13.0214 - 1834.8533x R = 0.99
7.2
7.1
7.0
6.9
6.8
ln (TENSILE STRENGTH)
0.0032
0.0033
0.0034
1/TEMPERATURE (°K)

TABLE 4: Average mesh-size of synthesized cross-linked copolymers (x-AA/MM)

PERCENT ACRYLIC ACID (%)	100	89.4	89.0	83.6	79.8	68.8	59.3	53.1
AVERAGE MESH-SIZE (A^{o})	155.1	149.7	143.3	130.3	131.8	112.7	99.6	90.2

Figure 38: Effect of acrylic acid composition on average mesh size of the copolymer network


R = 0.99
AVERAGE MESH SIZE (A°)
160
140
120
100
80
50
60
70
80
90
100
PERCENT ACRYLIC ACID

$$D = (\overline{\xi})^c \quad (17)$$

where c is a constant.

From equation (11) s^2 = constant x D

then s^2 = constant x $(\overline{\xi})^c$ (18)

$$2 \ln s = \text{constant} + c \ln \overline{\xi} \quad (19)$$

$$\ln s = \text{constant} + c/2 (\ln \overline{\xi}) \quad (20)$$

From equation (15) tensile strength = constant x s, therefore

$$\ln (\text{tensile strength}) = \text{constant} + c/2 (\ln \overline{\xi}) \quad (21)$$

A plot of ln (tensile strength) versus $\ln \overline{\xi}$ is shown in Figure 39, with correlation coefficient of 0.98, and a slope of 3. Therefore

$c/2 = 3$

$c = 6$

Therefore, for acrylic polymers, diffusion coefficient is proportional to average mesh size to the power of six, i.e.,

$$D = \text{constant} \times (\overline{\xi})^6 \quad (22)$$

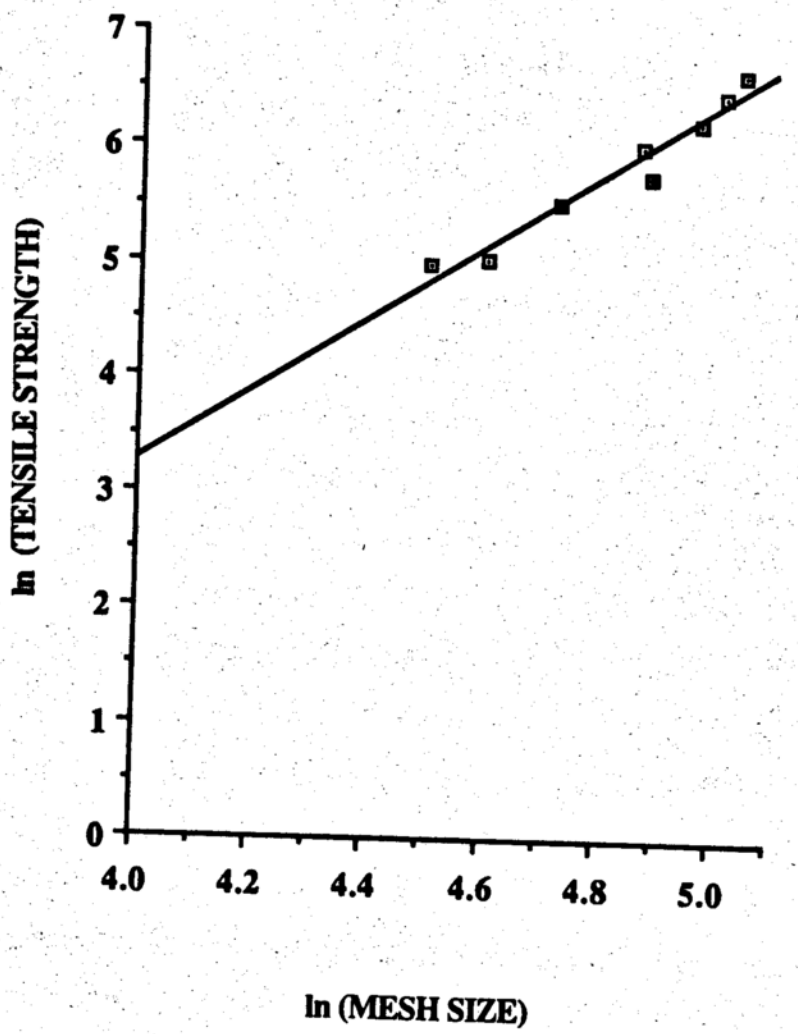
The relationship of tensile strength to mesh size of the acrylic polymer's network is

$$\text{tensile strength} = \text{constant} \times (\overline{\xi})^3 \quad (23)$$

Thus, mucoadhesive with desirable tensile strength can be designed by controlling the mesh size of the copolymer network.

Figure 39: A graph of ln (tensile strength) versus ln (mesh size)

y = - 9.0605 + 3.0852x R = 0.98



V. CONCLUSION

Bioadhesive polymers and/or copolymers have recently received considerable attention as platforms for controlled drug delivery. In using bioadhesives to prepare dosage forms, it is necessary to consider polymers or copolymers with both good adhesive strength and drug releasing profile. Thus, a better understanding of the structure-property relationship between bioadhesives and the mucin-epithelial layer is essential.

Mucus is a continuous network of cross-linked glycoprotein that covers most of the body orifices and the bladder. At physiological pH of 7.4, the mucin network carries a substantial negative charge due to the presence of sialic acid and sulfonic acid residues. The negative charge of the mucin network is particularly important for interaction with negatively or positively charged bioadhesives.

The bioadhesion process begins with establishment of intimate contact via viscoelastic deformation between the bioadhesive and substrate, followed by formation of secondary bonds, e.g., electrostatic and hydrophobic interactions, hydrogen bonding and van der Waals intermolecular interactions.

In the first stage of mucoadhesion, establishment of intimate contact, the surface characteristics and the composition of the mucoadhesive and mucin, and applied force or pressure are important parameters. The establishment of intimate contact can be enhanced by increasing the applied pressure, and interaction

between the adhesive's and substrate's surfaces. Thus, compatibility of the mucoadhesive and substrate favors the initial establishment of intimate contact.

In the second stage of bioadhesion, secondary bonds are formed between the adhesive and the mucin-epithelial surface. The important secondary bondings involves are hydrogen bonding, electrostatic and hydrophobic interactions.

In addition, the expanded nature of the mucin and polymer networks permit mutual interpenetration. Interpenetration/ interdiffusion of mucin and adhesives results in an increase in contacting area and establishment of physical entanglement of the two different macromolecules. Physical entanglement will strengthen the network in the interfacial area, whereas an increase in contact area will result in an increase in formation of secondary bonds. Thus, mucoadhesive strength can be increased by increasing the extent of interpenetration.

Tensile strength is shown to be a time dependent process. Thus, interpenetration is proposed to be a time dependent process, and adequate contacting time, e.g., two minutes, is required for sufficient interpenetration/interdiffusion to occur between acrylic polymer and mucin. Thus, one way of increasing the mucoadhesive strength is by increasing the time of contact between mucoadhesive and mucin substrate.

Tensile strength is also shown to be proportional to the mean diffusional path, i.e., depth of interpenetration of the

mucoadhesive and mucin networks. This helps explain the time dependency of tensile strength, since mean diffusional path or depth of interpenetration increases with time. secondary bonds for The importance of interpenetration/interdiffusion process in mucoadhesion suggests the importance of chain-segment mobility of the polymer and mucin chains. Less rigid polymer and mucin networks will increase the flexibility and ease of movement of both polymer and mucin chain. Increased flexibility and ease of polymer and mucin chains movement will decrease the activation energy of the process. The existence of an activation energy is supported by the temperature dependence of tensile strength. The experimental activation energy of interpenetration between mucin and acrylic polymer is found to be 30.5kJ/mole.

It is also concluded that increase in diffusion coefficient will increase tensile strength. The greater the diffusion coefficient, the greater will be the depth of interpenetration, and subsequently, greater will be the physical entanglement and contacting area for secondary bonds formation.

Furthermore, tensile strength was found to depend on the average mesh size, i.e., openness of the polymer network. An increase in the average mesh size, will increase the mobility of the macromolecular chains with subsequent increase in mucoadhesive strength. Thus, mucoadhesive with desired mucoadhesive strength can be designed by controlling the percent of charge groups and corresponding average mesh size of the network.

In conclusion, any factor that favors the intimacy of contact between mucoadhesive and mucin, or enhances the interpenetration process, or maximizes secondary bonds formation will potentially increase mucoadhesive strength. Polymers with flexible chains that form an expanded network and are compatible with mucin are favorable candidates to use as adhesives in developing mucoadhesive dosage forms. The expanded network can be formed chemically by cross-linking or physically by entanglement. Polymers that have secondary interactions with mucin will have stronger mucoadhesion, and thus are better mucoadhesives.

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