CHAPTER II

REVIEW OF LITERATURE

Pharmaceutical aspect of mucoadhesion have been the subject of great interest during recent years because a mucoadhesive drug delivery system has three desirable features (Jimenez-Castellanos, Zia and Rhodes, 1993; Lee, 1991):

1. Localization of the dosage form in specific regions to improve and enhance bioavailability of drugs.

2. Promotion of intimate contact of the formulation with the underlying absorbing surface to allow modification of tissue permeability or absorption of macromolecules, e.g., peptides and proteins.

3. Prolonged resident time of the dosage form.

Adhesion can be defined as the bond produced by contact between a pressure-sensitive adhesive and a surface. By another definition, it is the ability of an adhesive to another surface. It can also donate a state in which two surfaces are held together by interfacial forces, which may consist of valence forces and/or interlocking action.

Recently, bioadhesion is defined as the state in which two materials, at least one of which being of biological nature, are held together for an extended period of time by interfacial forces. By another definition, bioadhesion is the ability of a material (synthetic or biological) to adhere to a biological tissue for an extended period of time. The biological surface can be epithelial tissue or it can be the mucous coat on the surface of a tissue. If adhesive attachment was to a mucous coat, the phenomena would be referred to as mucoadhesion (Jimenez-Castellanos, Zia and Rhodes, 1993).

Mucous Layer (Duchene, Touchard and Peppas, 1988).

In most instances the mucoadhesive polymer is in contact with a soft tissue. Thus, the tissue layer responsible for formation of the adhesive interface is mucous. The term mucous usually refers to the layer covering the mucosa. It is synthesized by the globlet cells lining the mucosal epithelium layer and by special exocrine glands, such as salivary glands. It is a highly vicous liquid adhering to the epithelium. Its role is mucosa protection against various aggressions: mechanical, chemical, bacterial or viral. The understanding of bioadhesion requires knowledge of the mucous.

Chemical Composition of Mucous (Lee, 1991).

Beside water which represent more than 95 % of the mucous, the major components of the mucous are glycoproteins (0.5 to 5 %), lipids in

low proportions, mineral salts (1 %) and free proteins (0.5 to 1 %). The exact mucous composition varies depending on its source.

Glycoproteins are the main mucous components responsible for its viscosity and adhesive and cohesive properties. Basically glycoproteins consist of a protein core on which are attached oligosaccharide chains (diagram (a) in figure 1). Glucidic chains essentially contain galactose, N-acetylgalactosamine, N-acetylglucosamine, sialic acid and fucose. Linkages between the protein core are of the O-glucosidic type between N-acetylglucosamine and serine or threonine. Many of the terminal residues in the oligosaccharide side chains are sialic acids negatively charged at pH greater than 2.8 making the protein an anionic polyelectrolyte. Sulphate residues contribute equally to this negative charge. The mucous gel structure is the consequence of the intermolecular association of glycoproteins in a polymeric network. Previously thought to be a tetramer (diagram (b) in figure 1), the polymer is now believed to be a terminally linked chain with numerous crosslinkings. It has been proposed that chains result from disulphide bonds (interchain) and macromolecular associations are due to physical bonds stabilized by eletrostatic interactions (hydrogen bonding, salt linkage) or other non-covalent contacts between the oligosaccharide chains or between chains and the protein core of the molecule (figure 2).

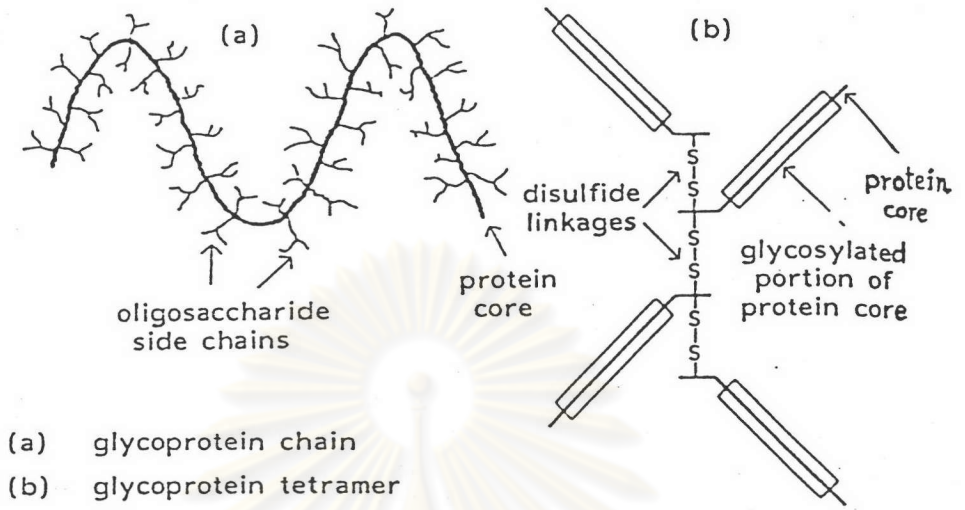


Figure 1: Schematic representation of the mucous.

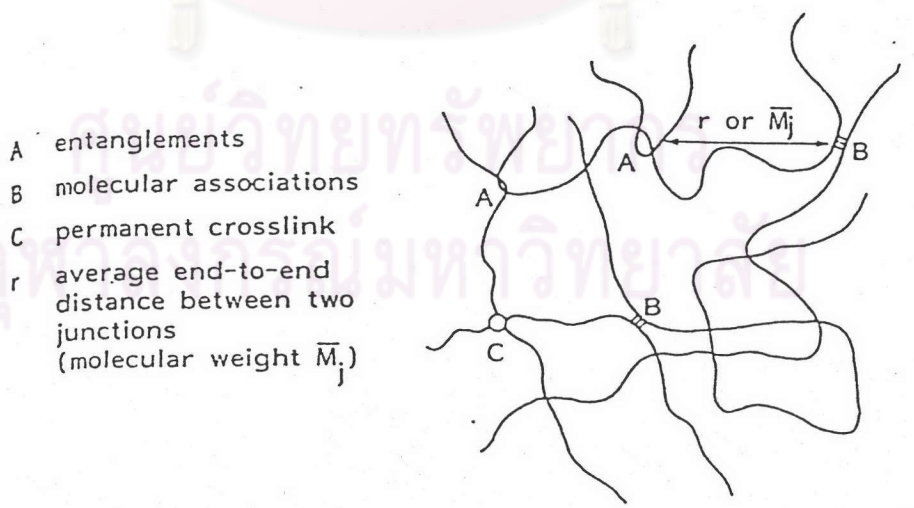


Figure 2: Crosslinked structure of the intestinal mucin.

Bioadhesion Mechanism (Duchene, Touchard, and Peppas, 1988).

The role of bioadhesion depends on the bioadhesive nature. For bioadhesion to occur, a succession of phenomena is required. Bioadhesion stages can be summarized as follows. First intimate contact must exist between the bioadhesive and the receptor tissue. This contact results either from a good wetting of the bioadhesion surface or from the swelling of the bioadhesive when contact is established. The penetration of the bioadhesive into the crevices of the tissue surface or interpenetration of bioadhesive chains with those of the mucous then takes place. Low chemical bonds can then settle (figure 3).

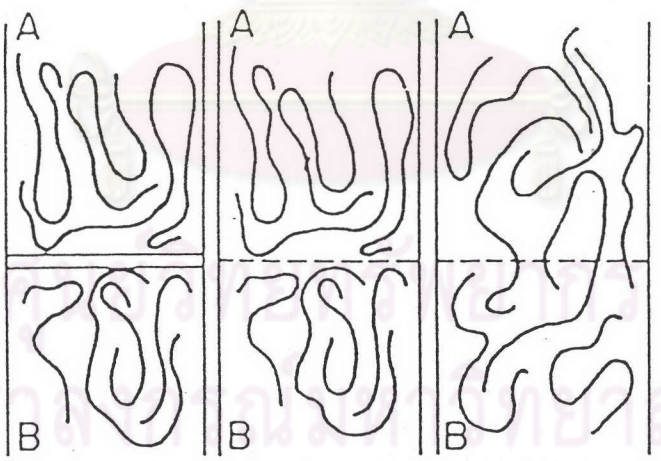


Figure 3: Chain interpenetration during bioadhesion of polymer A with the mucous B.

The intimate contact would occur if the bioadhesive material penetrated the crevices of the tissue on which it is applied. Hence the tissue surface roughness is an important factor for bioadhesion. A rough surface may be defined by the aspect ratio of maximum depth, d, to maximum width, h (figure 4). For adhesive purposes, the insignificant roughness occurs when the aspect ratio, d/h, has the value of less than 1/20. When the bioadhesive material is solid, its swelling in contact with moisture is necessary in order to impart sufficient freedom to the constituent chains.

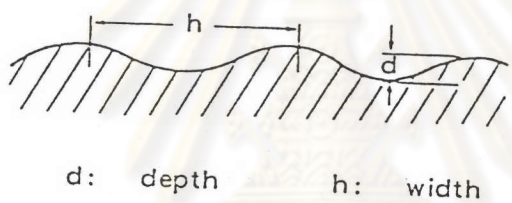


Figure 4: Surface roughness of soft tissue.

The interpenetration of chains from the bioadhesive polymer and mucous to a depth sufficient to create semi-permanent bonds corresponds to the diffusion theory. During chain interpenetration (figure 3), the molecules of the bioadhesive and the glycoproteinic network are brought into intimate contact and due to the concentration gradient, the bioadhesive polymer chains penetrate at rates which depend on the diffusion coefficient of a macromolecule through a crosslinked network and the chemical potential. With crosslinked polymers, the interpenetration of large chains occurs with greater difficulty. However, smaller chains and chain ends may still contribute to interdiffusion.

It is possible to determine the characteristic time for bioadhesion t by setting:

$$t = \frac{l^2}{D_b}$$

where l is the interpenetration depth and D_b is the bioadhesive material diffusion coefficient through the mucous.

The adhesion chemical bonds are of the primary or secondary type. The primary chemical bonds have a covalent nature of which high strength results in permanent bonds undesirable in bioadhesion. The secondary chemical bonds comprise a group of many different forces of attraction including electrostatic forces, Van der Waals forces, and hydrogen and hydrophobic bonds.

The electrostatic attractions are due to Coulomb forces between molecules of opposite charges. The Van der Waals forces are all the interactions between uncharged molecules attributed to three types of force: polar (or Keesom) forces resulting from the orientation of permanent dipoles in two molecules, induction (or Debye) forces arising from a permanent dipole in another molecule, and dispersion (or London) forces resulting from instantaneous changes in the charge distribution around non-polar molecules. The hydrogen bonding occurs when a specific hydrogen atom from one molecule is associated with another atom from a second molecule. The

hydrophobic bonding occurs when non-polar groups are associated with each other in an aqueous solution due to the tendency of water molecules to exclude the non-polar molecules. The secondary chemical bonding is the most important in bioadhesion.

The fracture theory of bioadhesion explaining the surface separation after bioadhesion attempts to relate the difficulty of separation of two surfaces after adhesion due to the adhesive bond strength.

Mucoadhesive Dosage Forms for Various Routes of Administration

(Lee, 1991).

A. Ocular Route: A good ocular mucoadhesive delivery system needs to control drug loss via the ocular drainage system.

B. Nasal Route: The nasal mucosa has a dense vascular network providing an excellent absorptive surface. The intranasal administration might be useful for many compounds which are not absorbed orally or suffer from extensive first-pass metabolism.

C. Buccal Route.

D. Gastrointestinal Route: A primary objective of using mucoadhesive formulations orally would be to achieve a substantial increase in length of stay of the drug in the gastrointestinal tract.

E. Colonic and Rectal Routes.

F. Cervical and Vaginal Routes.

The mucoadhesive dosage forms for buccal route have been the topic of great interest during recent years. These forms can be summerized as follows.

Mucoadhesive Dosage Forms for Buccal Route.

The total area of the buccal cavity is about 100 cm^2. The mucosal surface is constanly washed by 0.5-2 l of saliva daily. The buccal route offers the advantages of lower enzymatic activity and avoidance of first-pass metabolism and gastric enzymatic degradation. The oral cavity allows precise localization of a mucoadhesive drug delivery system and removal of the formulation if and when irritation occurs. Localization of the formulation allows the addition of an absorption enhancer to modify the underlying absorbing epithelium to enhance drug availability.

The mucoadhesive dosage forms for buccal route may be catagorized into tablet, ointment or patch.

1. Tablet.

Insulin mucoadhesive tablet (Ishida, et al., 1981) was prepared to solve the problems of the administration of insulin by injection (figure 5). A mixture of hydroxypropylcellulose (HPC) and carbopol 934 (CP 934) was used in this new form. Unfortunately, the percentage of insulin absorbed from this dosage form was about 0.5% compared with the amount absorbed through intramuscular injection of insulin.

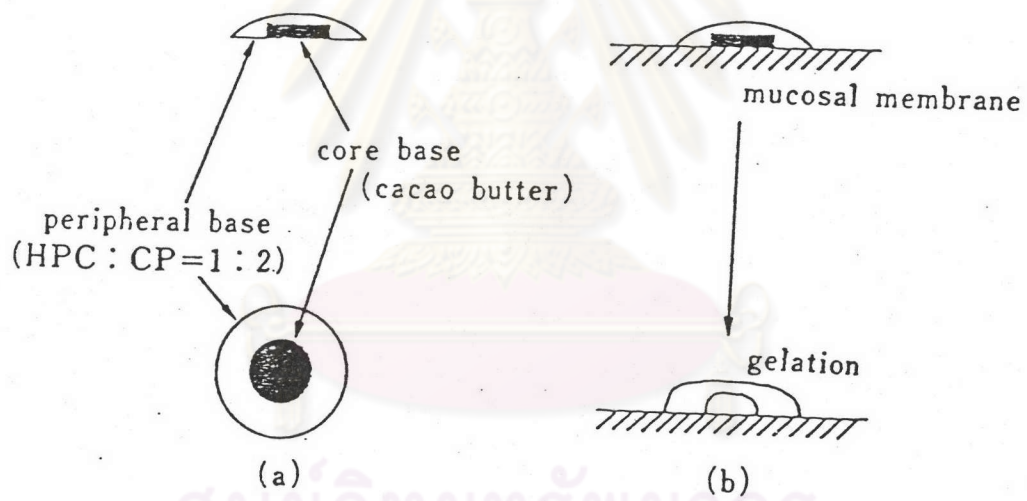
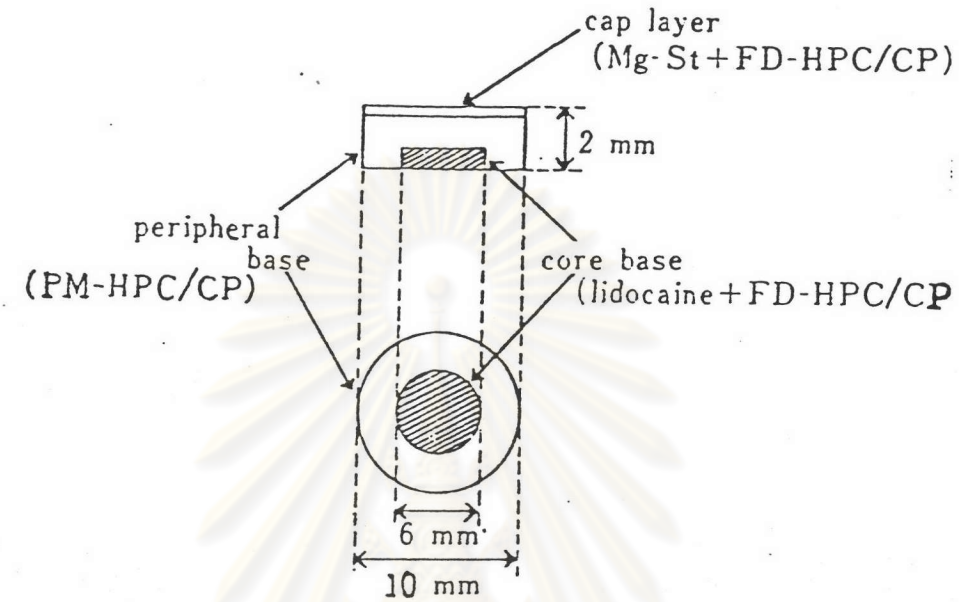


Figure 5: Bioadhesive device of insulin.

Lidocaine mucoadhesive tablet (Ishida, Nambu and Nagai, 1982) was developed for toothaches. This preparation contained lidocaine, HPC and CP934 (figure 6). They were covered with a freeze dried mixture of HPC, CP934 and magnesium stearate. This dosage form could afford a prolonged local anesthetic action.



F D = Freeze dried mixture

P M = Physical mixture

Figure 6: Bioadhesive device of lidocaine.

Triamcinolone tablet (Duchene, Touchard and Peppas, 1988) was formulated using the principles of mucoadhesion for the treatment of aphthous stomatitis (figure 7). It was a double layer tablet. The upper coloured layer was lactose and has no adhesive property; its roles were to prevent active ingredient (triamcinolone acetonide) diffusing out of its active site and to allow an easy placing of the tablet. The lower layer which contained the active ingredient was made of HPC and CP934 and constituted the bioadhesive layer. This tablet was commercially available under the name of 'Aftach'.

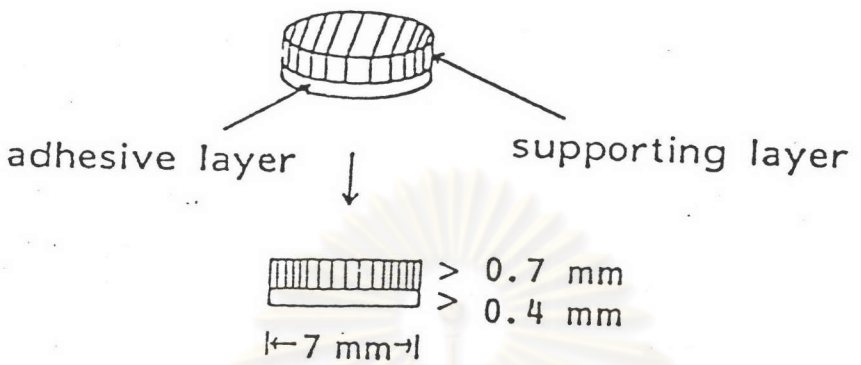


Figure 7: Bioadhesive device of triamcinolone.

Nitroglycerin mucoadhesive tablet (Schor, et al. 1983) consisted of polymers made from naturally occurring materials (Synchron^{R}) which could be mixed directly with an active pharmaceutical substance and directly compressed into tablets for the treatment of angina pectoris. The tablet completely dissolved over a period of hours to produce a steady, high level of clinical activity over a period of 5 to 6 h.

Timolol mucoadhesive tablet (Deasy and O'Neill, 1989) was developed to solve the problem of conventional oral treatment that is large individual variation of absorption (figure 8). The core contained timolol and glycerol palmitostearate. HPC and CP 934 were the bioadhesive polymers. The cap layer was magnesium stearate. The result in human showed that an average of 34 % of the drug loading was absorbed in an appearently zero order manner over 3 h. The addition of 0.1% sodium lauryl sulfate to the

tablet enhanced penetration increasing the mean quantity absorbed over 3 h to 61 % of the drug loading.

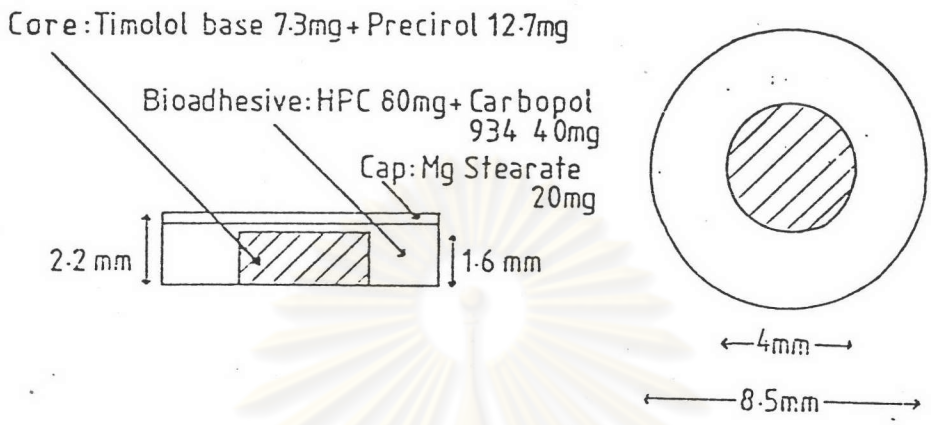


Figure 8: Bioadhesive device of timolol.

Cetylpyridinium chloride mucoadhesive lozenge (Collins and Deasy, 1990) was developed to treat oral infection (figure 9). It was a three layered device by filling the desired proportions of the components (spermaceti wax, CP 934, flavouring agent, HPC, magnesium stearate, precirol and talcum). The device offered considerable improvement over the proprietary product in sustaining salivary levels.

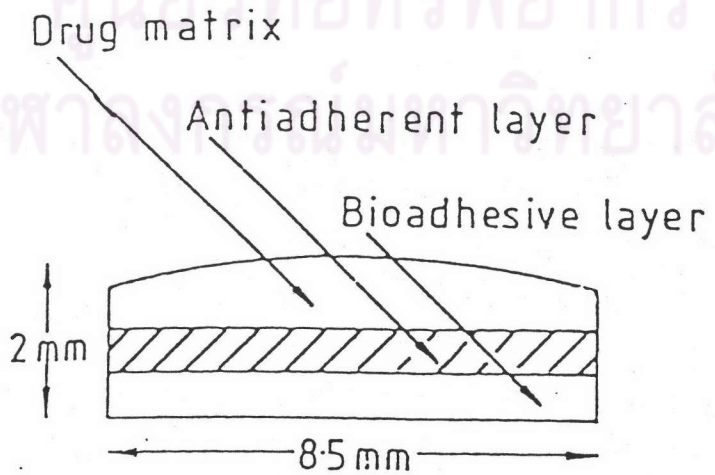


Figure 9: Bioadhesive device of cetylpyridinium chloride.

Fluoride mucoadhesive tablet (Bottenberg, 1991) was designed for the prevention of dental caries. As recent studies have shown that fluoride is effective in small concentrations when it is present long enough in the fluid surrounding the enamel; an oral slow-release fluoride administration seems to be the method of choice. The experiment concluded that bioadhesive polymers such as thermally modified corn starch with 5 % polyacrylic acid (CP 934) or polyethylene glycol (M.W. 300,000) could be used as a slow-release device for fluoride. And it could be shown that the bioadhesive slow-release tablet offered an effective way of sustaining a fluoride level in saliva *in vivo*. Other topical fluoridation products such as gel mouthrinses or toothpaste achieved high salivary levels for a short period. Also, conventional fluoride tablets did not achieve a prolonged release (Bottenberg, 1992).

2. Ointment.

Prednisolone mucoadhesive ointment (Ishida, et al., 1983) was developed for the treatment of aphthae using CP 934 as the mucoadhesive agent. The investigation showed that the release of prednisolone from the ointment-type oral mucosal dosage form containing 30 % CP 934 was better than the original base.

Tretinoin mucoadhesive ointment (Bremecker, Strempel and Klein, 1984) consisting of neutralized polymethacrylic acid methyl ester was

formulated to treat lichen planus and reduce irritation to the mucous membranes.

3. Patch and Film.

An application of lignocaine mucoadhesive patch to the oral mucosa produced soft tissue anaesthesia and reduced systemic effect (Brook, et al. 1989).

In 1989 Anders and Merkle prepared two-ply laminates of an impermeable backing layer and a mucoadhesive polymer layer containing the drug.

Other oral adhesives used in the mouth were developed in dental practice. The active ingredient was antiseptic or antimicrobial agents such as chlorhexidine and metronidazole (Steinberg and Friedman, 1988).

Method to Screen and Evaluate Mucoadhesion.

Over the past decade, considerable effort has been devoted to establish suitable screening to evaluate mucoadhesives. Various methods for studying mucoadhesion have been described and can be classified into two groups: a) *in vitro* methods; most of which require the use of an artificial biological medium such as mucous or saliva and are based on either tensile strength or shear strength measurements (Lee, 1991). And b) *in vivo* methods.

a) *In vitro* methods.

Shearing stickiness test of ointment (Ishida, et. al., 1983): The thickness of ointment applied between two glass plates was 0.3-0.4 mm.. A string was wound and the value of shearing stickiness was represented by the reading on the spring balance (figure 10).

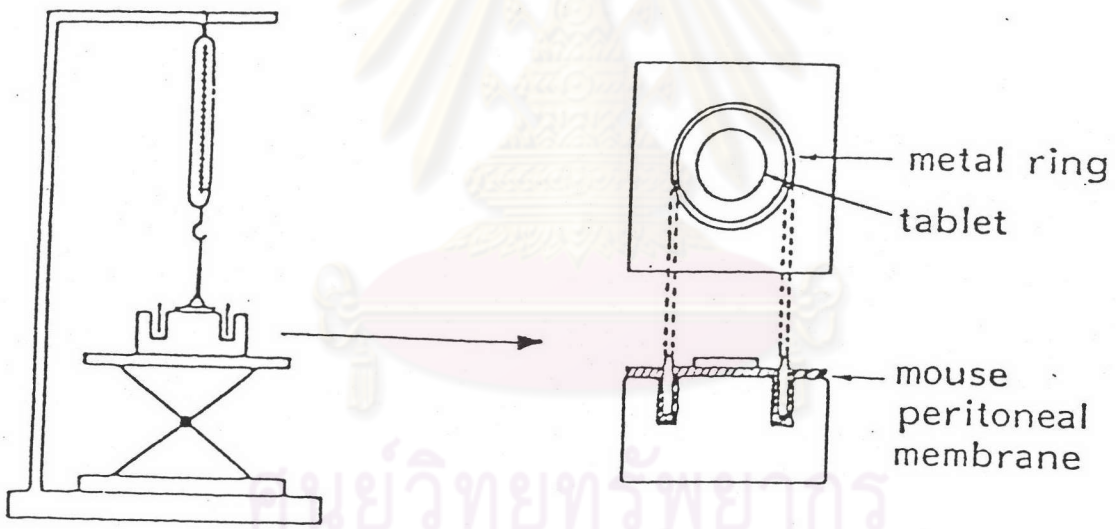


Figure 10: Diagrammatic representation of Ishida et. al. (1983 a) for determining bioadhesive tensile strength.

Wilhelmy plate method modification (Smart, Kellaway and Worthington, 1984): In this method the plates were coated with the polymer to be tested and immersed in a temperature-controlled mucous solution. The

force required to detach the glass plate coated with the test material was measured (figure 11).

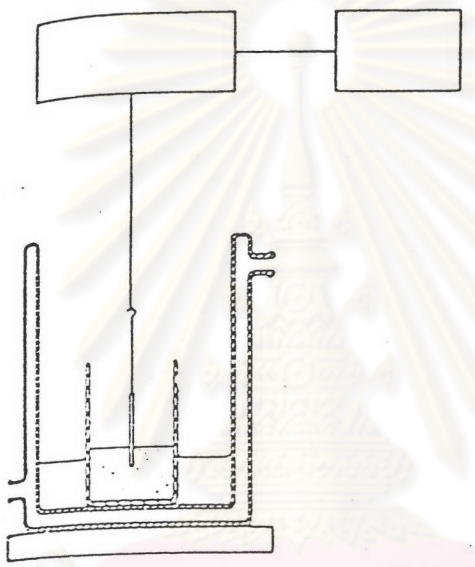


Figure 11: Diagrammatic representation of Smart and Kellaway (1984) for determining bioadhesive tensile strength.

In 1991 Chitnis, Malshe and Lalla modified the study of adhesion of the polymer which was based on Wilhelmy plate (figure 12). A resin sheet coated with the test material was slowly pulled away from the mucin, and then the minimum weight displayed was recorded.

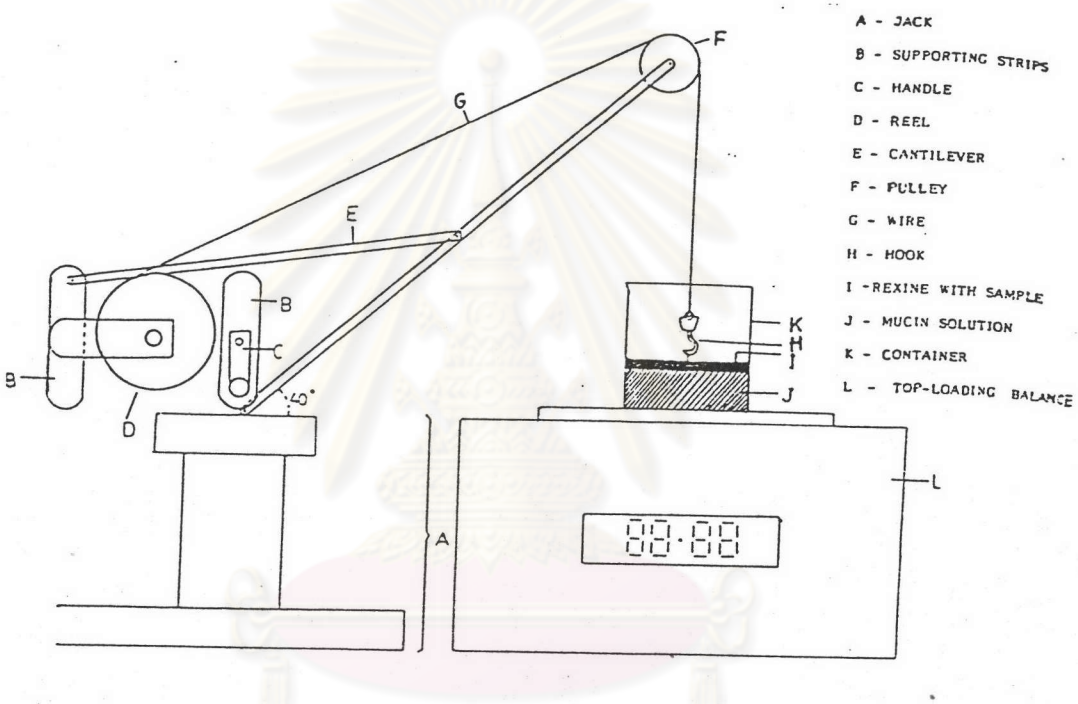


Figure 12: Diagrammatic representation of Chinis et. al., (1991) for determining bioadhesive tensile strength.

Stickiness test (Ishida, 1981): The preparation was stuck to the mouse peritoneal membrane and the sticking force was measured (figure 13).

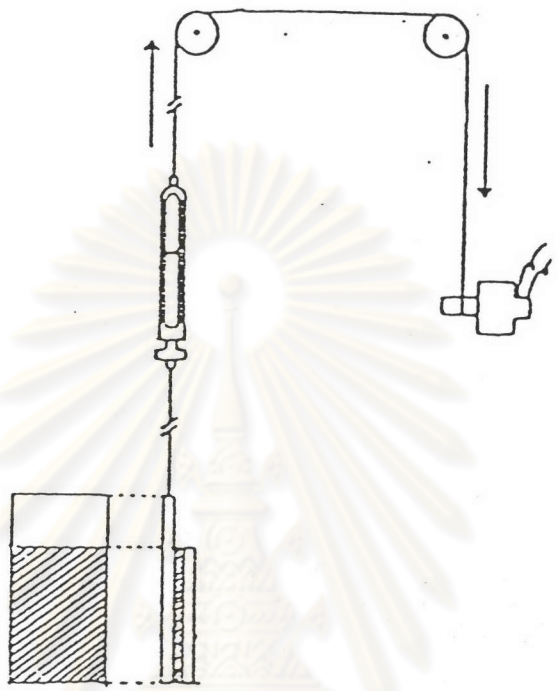


Figure 13: Diagrammatic representation of Ishida et. al. (1981) for determining bioadhesive tensile strength.

Dual tensiometer (Leung and Robinson, 1988): This apparatus was designed to measure shear stress in mucoadhesion (figure 14). Part A is the regular modified tensiometer. The mucosa is secured on the top and bottom holders. The surface of the top holder is coated with the hydrated test polymer. Part B is a second tensiometer to measure the strength.

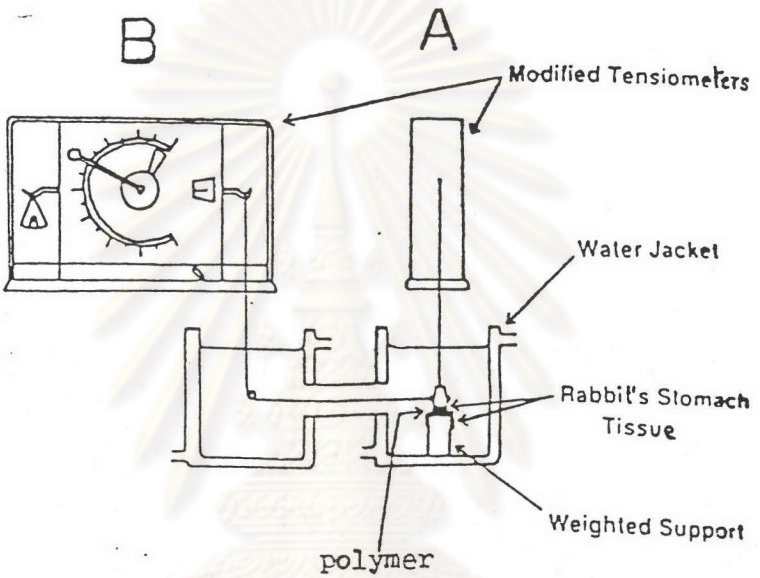


Figure 14: Diagrammatic representation of Leung and Robinson (1988) for determining bioadhesive tensile strength.

Tensile tester (Lejoyeux, 1989): The apparatus is illustrated in figure 15. The mucosa was stuck on the lower support and the test tablet was fixed on the upper support. The detachment force was recorded as a function of displacement up to the total separation of the two surfaces.

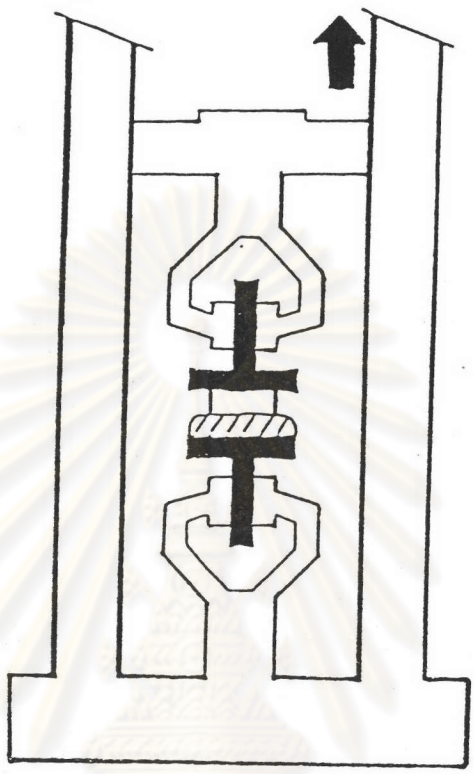


Figure 15: Diagrammatic representation of Lejoyeux et. al., (1989) for determining bioadhesive tensile strength.

Another method that proposed to measure mucoadhesion illustrating in figure 16. The section of mucous tissue was fixed on the lower support and the test preparation was stuck onto the lower surface of the weight. The platform was lowered until the upper and lower surfaces separated from each other and the force at the adhesive bond failed to record.

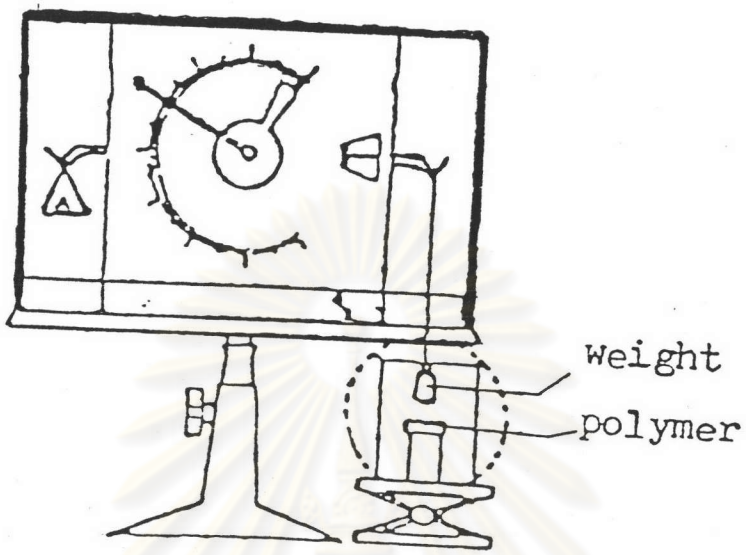


Figure 16: Diagrammatic representation of Ch'ng et. al. (1985) for determining bioadhesive tensile strength.

b) *In vivo* method.

This method used male Sprague Dawley rats (Jimenez- Castellanos, Zia and Rhodes, 1993). A capsule containing test material was surgically inserted into the stomach of anesthesized rats. The rats were permitted to awaken and at suitable times the animals were sacrificed, and the stomach and small intestine were removed. The radioactivity was measured in each segment of the stomach and intestine.

Polymer-Related Factors Affecting Bioadhesion.

The bioadhesive power of a polymer or of a polymers series is affected by the polymer nature.

The structure, molecular weight and swelling property of polymer affect the bioadhesive force (Ponchel, 1987; Mikos and Peppas, 1990; Bodde, De Vries, Junginger, 1990; Leung, 1990; Chu, et al., 1991). It seems that the bioadhesive force increases with the molecular weight of the bioadhesive polymer up to 100,000. Beyond this level there is not much effect. But it is also necessary to consider the size and configuration of the polymer molecule. Hence, for example, with polyethylene oxide, the adhesive strength increases even up to the molecular weight of 4,000,000; these polymers are linear configuration. On the other hand, with dextran molecules with molecular weights as high as 19,500,000 do not exhibit better bioadhesion than molecules with molecular weight of 200,000.

Many previous studies used a mixture of CP 934 and HPC (Ishida, et. al., 1981; Ishida, et al., 1982) and a mixture of CP 934 and HPMC (Duchene, et al., 1982). In both cases, CP 934 was the bioadhesive agent and the cellulosic derivatives were the hydrophilic matrices.

The bioadhesive power is also affected by the concentration of active polymer. The bioadhesive force increases with the bioadhesive

polymer concentration in solid dosage forms such as tablet (Ponchel, et al., 1987).

Evaluation of the Adhesive Behavior.

Previous studies were *in vivo* experiments using humans (Collins and Deasy, 1990; Bottenberg, 1992; Brook, 1989) and dogs (Ander and Merkle, 1989; Deasy and O'Neill, 1989). The volunteers were asked to note the retention time of the preparation and remark about the degree of irritation or discomfort. In the observation of lignocain patch (Brook, 1989), the test buccal area was indistinguishable from the surrounding mucosa. Anders and Merkle (1989) evaluated the laminated mucoadhesive patches for buccal. They showed the effects of polymer species and viscosity grades to the duration of mucosal adhesion of the adhesive patches.

Release of Active Agent.

The experiments were carried out either *in vitro* (Bottenberg, 1992; Ishida, Nambu and Nagai, 1982; 1983a; 1983b; Deasy and O'Neill, 1989) or *in vivo* (Collins and Deasy, 1990; Anders and Merkle, 1989; Deasy and O'Neill, 1989). The *in vitro* and *in vivo* releases of timolol from bioadhesive tablet which was prepared by Deasy and O'Neill (1989) appeared to be zero order. The difference in the release of each preparation depended on the polymer species and the percentage of polymer in the preparation (Ishida, Nambu, and Nagai, 1982; 1983b). The release was delayed as the

percentage of CP 934 was increased thus, the preparation would stick more tightly and for a longer time.

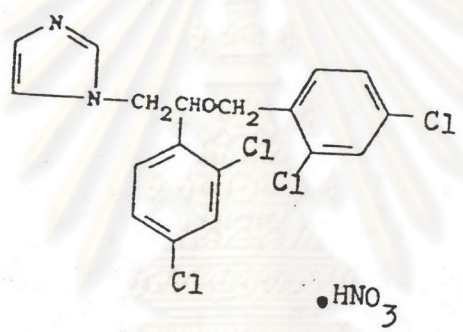
Stability Testing of Drug Product.

It is difficult to find a completely general rule for stability testing of new preparations. However, there are some general method in "stability testing of drug product" (Grimm, 1987) and "drug stability principles and practices" (Carstensen, 1990). The test is performed to estimate by a short-term test which is the quality of a drug in a certain period of distribution. When a partial modification of a finished product is intended, the result of relative comparative test of the finished products before and after the modification may be accepted. In the stability testing of a solid drug product, the relative comparative test shall be performed for three months or more, in principle, at 40°C (±1°C), 75 % RH (±5 %). The result obtained at 40 °C, 75% RH for one month corresponded to those at 25°C, 75% RH about one year. In the"drug stability principles and practices", most tests have been performed at 37-40°C, 75-100% RH for three months (the Joel Davis test). The result of this test is often how FDA justifies granting a two-year expiration period.

Miconazole Nitrate

(Reynolds, 1993; Rockvillie, 1990)

Miconazole nitrate is a white or almost white, odourless or almost odourless, crystalline or microcrystalline powder. Very slightly soluble in water and ether; slightly soluble in alcohol and chloroform. The molecular structure is demonstrated below.



The empirical formula is $C_{18}H_{14}Cl_4N_2O.\ HNO_3$ with a molecular weight of 479.1.

Antimicrobial Action:

Miconazole is an imidazole antifungal agent with ergosterol synthesis and therefore alters the permeability of the cell membrane of sensitive fungi. It also has the *in vitro* activity against *Cladosporium, Madurella* and *Phialophora spp.* and against *Pseudallescheria boydii,* and Gram-possitive bacteria including staphylococci and streptococci.

Pharmacokinetics and Metabolism:

Miconazole is incompletely absorbed from the gastrointestinal tract: peak plasma concentrations of about 1 mcg per ml have been achieved at 4 h after a dose of 1 g. By intravenous infusion, doses above 9 mg per kg body-weight usually produce plasma concentrations above 1 mcg per ml. Miconazole has a terminal half-life of about 24 h. Over 90% is reported to be bound to plasma protein.

Miconazole is metabolised in the liver to inactive metabolites. 10 to 20% of an oral or intravenous dose is excreted in the urine, mainly as metabolites, within 6 days; about 50% of an oral dose may be excreted mainly unchanged in the feces. There is little absorption through skin or mucous membranes when miconazole nitrate is applied topically.

Indication:

Miconazole is administered by intravenous infusion in the treatment of severe systemic fungal infections including candidiasis, cryptococcosis, coccidioidomycosis, paracoccoidioidomycosis, and an infection due to *Pseudallescheria boydii*. It may be given by mouth as tablets or gel for treatment of oral and intestinal candidiasis. It has also been given prophylactically to patients at high risk of opportunistic fungal infections. For treatment of oral lesions the tablets are dissolved in the mouth; a 2% w/w oral gel may also be used. 2% Miconazole nitrate cream, lotion or

powder is applied for treatment of fungal infection of the skin and nails including candidiasis, tinea, and pityriasis versicolor.

Adverse Effects:

After the intravenous infusion of miconazole, phlebitis, nausea, vomiting, diarrhoea, anorexia, pruritus, rash, febrile reactions, flushes, drowsiness, and hyponatraemia have been reported. Effects on the blood include hyperlipidaemia, aggregation of erythrocytes, anaemia, and thrombocytosis. Transient tachycardia and cardiac arrhythmias have followed the rapid intravenous injection of miconazole.

Preparations:

10 mg/ml Miconazole injection.
2% W/W Miconazole nitrate oral gel.
2% W/W Miconazole nitrate cream.

Analysis of Miconazole:

A conventional UV spectrophotometry method was subject to possible interference by formulation excipients (DiPietra, et al., 1988). The analytical procedures for high performance liquid chromatography (HPLC) determination of miconazole were reported such as the method of Nishikawa and Fujii (1991) and the method of Tyler and Genzale (1989). The HPLC conditions of Nishikawa and Fujii were as follows: the apparatus used was

Water model 600 multi-solvent delivery system, model 490 variable wavelength UV detector and model 741 data analyzer, column μ Bondasphere 5 μ C_{18}-100 Å 3.9x150 mm. maintained at 40 °C; the mobile phase was 0.05 M ammonium dihydrogenphosphate-methanol (15:85, V/V), flow rate of 0.7 ml/min; and the detector wavelength of 272 nm. Clotrimazole was used as an internal standard. The spectrum of miconazole was obtained using a Hewlett-Packard Model 1040 A diode array system (figure 17). Tyler and Genzale selected the wavelength of 214 nm and used the fixed-wavelength zinc lamp detector.

Other reports were determination of miconazole in plasma (Sternson, Patton and King, 1982) and saliva (Turner and Warnok, 1982).

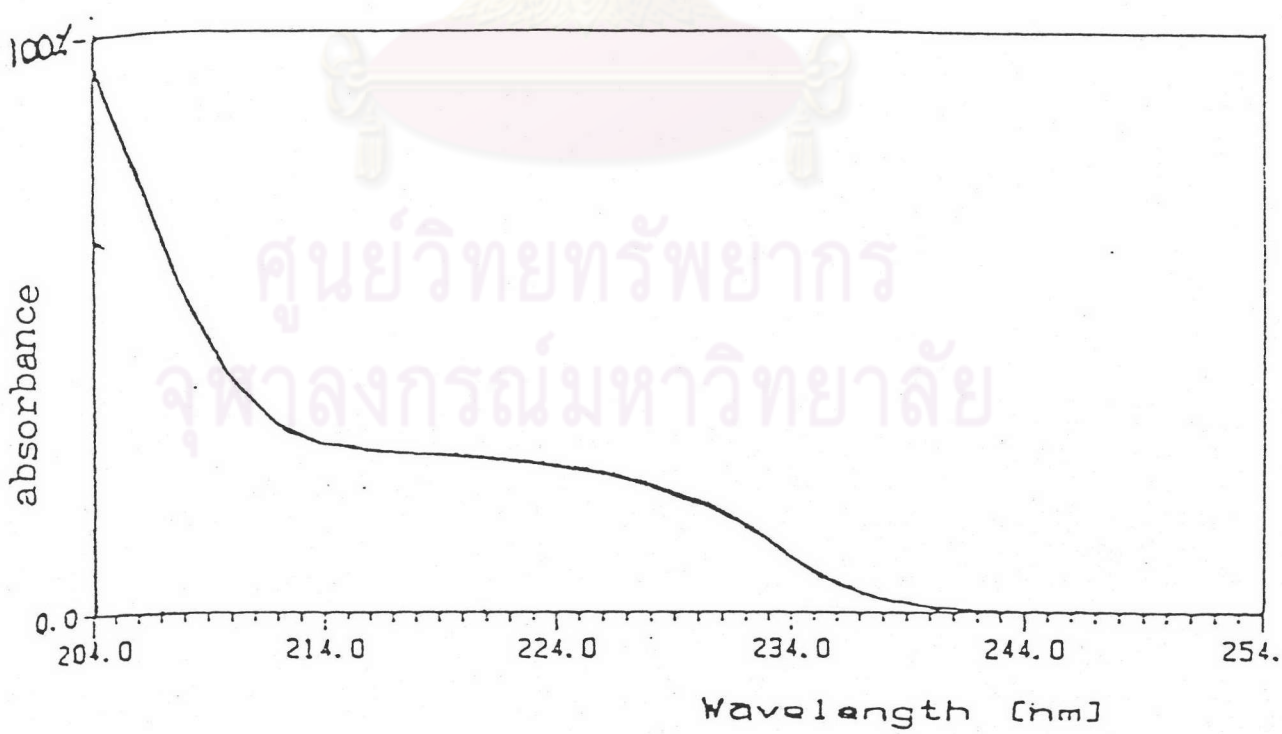


Figure 17: Absorption spectrum of 0.80 mg % miconazole.