CHAPTER II
LITERATURE REVIEW

I Botanical, Chemical and Pharmacological Aspects of *Garcinia mangostana* Linn.

1 Botanical aspects of *Garcinia mangostana* Linn.

Garcinia mangostana Linn. is a tree, 7-12 m high, having straight trunk, brown to blackish bark, young branch quadrangular, exuding yellow gum-resin. Leaves are opposite, ovate or elliptic – oblong, 6-11 cm wide, 15-25 cm long, with dark green and glossy above, yellowish green below. Flowers are solitary or in pairs near the twig ends, yellowish green with red edges or almost entirely red. Fruit is globose 4-7 cm in diameter, having short and thick stalk, dark purple with four persistant sepals at the base (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542; Promjit Saralamp et al., 1996; Farnsworth, and Bunyapraphatsara, 1992).

Garcinia mangostana Linn. is in the family of Guttiferae, its common name is mangosteen. It is a slow-growing tree which usually propagate by seeds and mostly proliferates in hot and humid climate, preferably with a short dry season such as in India, Thailand, Indonesia and Philippines (Farnsworth, and Bunyapraphatsara, 1992; Perry, and Metzger, 1980).

2 Chemical components of *Garcinia mangostana* Linn.

The chemical studies on the constituents of the fruit rind of *Garcinia mangostana* Linn. have revealed that the major substances are xanthones, the others component are cathecol triterpinoid, benzophenone (maclurin) and anthrocyanin glycosides (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542; Farnsworth and Bunyapraphatsara, 1992; Perry, and Metzger, 1980).

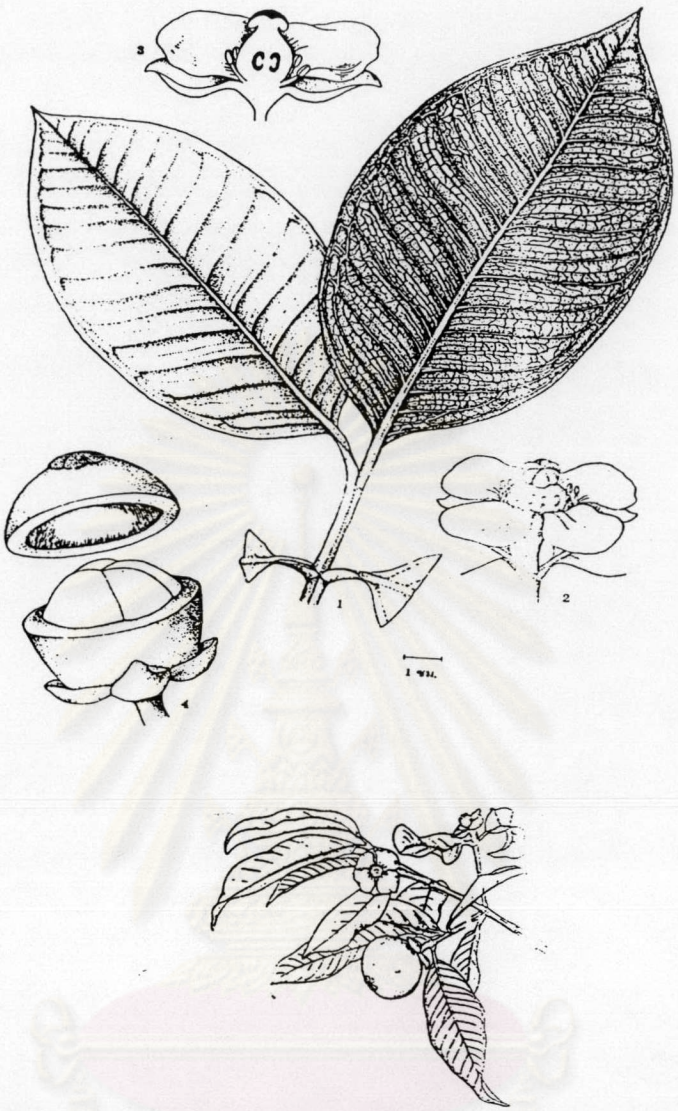


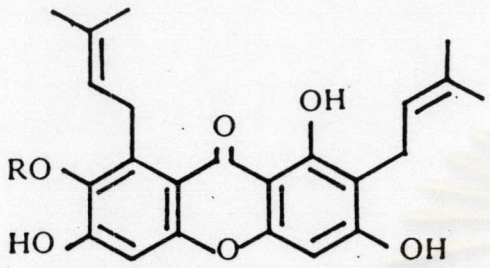
Figure 1 *Garcinia mangostana* Linn.

Xanthones are a restricted group of plant phenols. They are formed by the condensation of a phenylpropanoid precursor. Hydroxyxanthones are mainly found in Guttiferae and Gentianaceae. Xanthones show considerable biological activities such as strong antimicrobial, antiinflammatory and antitumor activities (Bruneton, 1995).

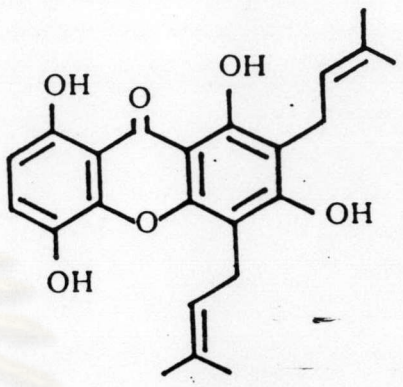
The major xanthones from fruit rind of *Garcinia mangostana* are mangostin α, β and γ, gratanin, 8-deoxygartanin and garcinones A, B, C and E, all of them have mono or diprenylated skeleton (นันทวัน บุณยประภัศร, 2533;Wilawan Mahabusarakam, Pichaet Wiriyachitra, and Saowaluk Phongpaichit, 1986; Govindacharit et al., 1971; Sen et al.,1980,1982)

Mangostin (1, 3, 6-trihydroxy-7-methoxy-2, 8,-bis-(3-methyl-2-butenyl)-9H-xanthone) is the most active component. Isolated from fruit rind by extracted with hexane or benzene, it appears as yellow crystals with melting point of 181.6-182.6 and molecular weight of 410.47. It is soluble in alcohol, acetone, chloroform and ethyl acetate but practically insoluble in water (นันทวัน บุณยประภัศร, 2533 ; Wilawan Mahabusarakam, and Pichaet Wiriyachitra, 1987).

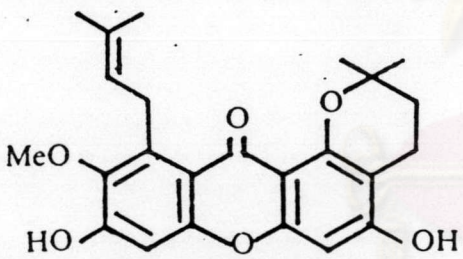
Moreover, various chemical compounds from fruit rind were reported as follows: isomangostin, normangostin, chrysanthemin, mangostin–3,6–di-O-glycoside, cyanidin–3–O–β–D–sophoroside, kolanone, 1, 5–dihydroxy–2–isopentenyl-3-methoxy xanthone, 1, 7–dihydroxy–2–isopentenyl-3–methoxy xanthone 5, 9–dihydroxy–8–methoxy–2, 2–dimethyl-7–(3–methylbut–2–enyl) 6 (H)–pyrano–(3, 2, 6)–xanthone – 6 –one, 3 – O – methyl mangostin, n–triacontane, mangostin triacetate, D–fructose, D–glucose (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542; Farnsworth, and Bunyapraphatsara, 1992).



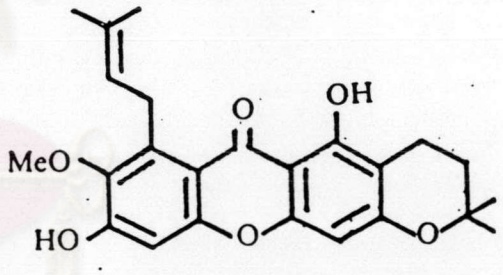
(1) R = Me, mangostin
(3) R = H, γ-mangostin



(2) gartanin



(4) 1-isomangostin



(5) 3-isomangostin

Figure 2 Some important chemical components in *Garcinia mangostana* Linn.

3 Pharmacological activities and toxicities of *Garcinia mangostana* Linn.

3.1 Pharmacological activities

There are many investigations on pharmacological activities of xanthones from *Garcinia mangostana* Linn.

3.1.1 Antimicrobial activity

Mangostin and its derivatives have an intense antimicrobial activity against both methicilln–resistant and methicillin–sensitive *Staphylococcus aureus* nearly equal to vancomycin (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542. นันทวัน บุญยประภัศร, 2533; เสาวลักษณ์ พงษ์ไพจิตร และคณะ, 2537; Wilawan Mahabusarakam, Pichaet Wiriyachitra, and Saowaluk Phongpaichit, 1986; Farnsworth, and Bunyapraphatsara, 1992). Moreover, the activity against other bacteria such as *Shigella dysenteriae, Shigella sonnei, Escherichia coli, Salmonella typhimurium, Vibrio cholerae, Pseudomonas aeruginosa and Bacillus subtilis* have been reported. (นันทวัน บุณยประภัศร, 2542; Farnsworth, and Bunyapraphatsara, 1992; Sundaram et al., 1983).

3.1.2 Antiinflammatory activity

Mangostin and its derivatives exhibited antiinflammatory activity both by intraperitoneal and oral administration in normal and bilaterally adrenalectomized rats when tested by the carageenan–induced pedal edema, cotton pellet implantation and granuloma pouch technique (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2533, 2542; Farnsworth, and Bunyapraphatsara, 1992)

3.1.3 Anti–ulcer activity

Mangostin produced significant anti–ulcer activity in rats (นันทวัน บุณยประภัศร, 2533, 2542; Frunsworth, and Bunyapraphatsara, 1992).

3.1.4 Antihistamine and antiserotonin activities

Mangostin presented a thermostable antihistamine and antiserotonin activities by reduced the contractions of isolated rabbit aorta which induced by histamine and serotonin. Furthermore, mangostin also exhibited the same activity as chlorpheniramine (Chirungsrilerd et al., 1996).

3.1.5 Antifungal activity

Mangostin and its derivatives showed the activity against *Trichophyton mentagrophytes, Microsporum gypseum, Alternaria solani, Cunninghamella echinulata* and *Epidermophyton floccosum* (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542 ; Farnsworth, and Bunyaphraphatsara, 1992; Sundaram et al., 1983).

3.1.6 Central nervous system depressant activity

Mangostin and its derivatives produces CNS depressant characterized by ptosis, sedation, decreased motor activity in mice and rats (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2533, 2542; Farnsworth, and Bunyaphraphatsara, 1992).

3.1.7 Cardiovascular effect

Mangostin–3,6–di–O–glucoside exhibited significant effects on the cardiovascular system of frogs and dogs by produced myocardial stimulation and increased the blood pressure (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542; Farnsworth, and Bunyaphraphatsara, 1992).

3.1.8 Inhibitory effects on HIV–1 protease and viral reverse transcriptase

Mangostin showed potent inhibitory activity against HIV–1 protease and viral reverse transcriptase (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542; Chen, Wan, and Loh, 1996).

3.1.9 Other activities

Mangostin and its derivatives also exhibited many activities such as antioxidant, antitumor and antimutagenic activities (นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542).

3.2 Toxicities of *Garcinia mangostana* Linn.

อัมพร ศรประสิทธิ์ และคณะ (2530) reported about acute toxicities of mangostin when treated the rats with a high dose of mangostin (1.5 g/1 kg body weight oral forced fed) compared with paracetamol(1.5 g/1 kg body weight). It was found that paracetamol increased the activities of serun glutamic –oxaloacetic transaminase (SGOT) and serum glutamic–pyruvic transaminase (SGPT) much more than mangostin and the amount of total liver protein of paracetamol treated rats decreased significantly, whereas mangostin did not change total liver protein. In summary, mangostin is safer than paracetamol.

It has been reported that the application of 1.5 percent *Garcinia mangostana* cream in the patients with chronic ulcer, produced no harmful side effects, allergic conditions and irritation (วัฒนีย์ ปานจินดา, 2535).

4 The medicinal uses of *Garcinia mangostana* Linn.

Garcinia mangostana Linn. has traditionally been used for a long time for the treatment of wounds and diarrhoea. The medicinal uses are as follows:

1. Root may be treated for irregular menstruation.
2. Bark has been used for washing and healing wounds, treatment of aphthous ulcer and diarrhoea.
3. Leaves are useful for treatment of dysentery.
4. Fruit rind is the astringent and has been used for treatment of both normal and infectious wounds include aphthous ulcer, it was also used for treatment of diarrhoea and dysentery (ก่องกานดา ชายามฤต, 2540; นันทวัน บุณยประภัศร และ อรนุช โชคชัยเจริญพร, 2542; Perry, and Metzger, 1980).

As the traditional recipes of *Garcinia mangostana* Linn. in treatment of diarrhoea, the dried fruit rind was boiled with water or saturated calcium hydroxide solution and the extract is taken. For treatment of wounds, the dried fruit rind was rubbed with saturated calcium hydroxide solution as a solvent the suspension was applied over the wound area (Farnsworth, and Bunyapraphatsara, 1992).

II. Oral Mucosal Ulceration Diseases

Ulceration of oral mucosa is a local defect of the surface, in which the covering epithelium is destroyed leaving an inflamed area of exposed connective tissue.

1. Classification of oral mucosal ulceration on an aetiological basis

1.1 Infective

1.1.1 Bacterial

1.1.2 Viral

1.1.3 Fungal

1.2 Traumatic

1.2.1 Mechanical

1.2.2 Chemical

1.2.3 Thermal

1.2.4 Radiation

1.3 Idiopathic

Recurrent oral ulceration (Recurrent aphthous ulceration)

1.4 Associated with systemic diseases

1.4.1 Haematological diseases

1.4.2 Gastrointestinal tract diseases

1.4.3 Other diseases

1.5 Associated with dermatological diseases

1.5.1 Erosive lichen planus

1.5.2 Vesiculobullous diseases

1.6 Neoplastic

1.6.1 Squamous cell carcinoma

1.6.2 Other malignant neoplasms

Recurrent oral ulceration and lichen planus are the most common oral mucosal disease(Cawson ,and Odell, 1998)

2. Recurrent oral ulceration (Recurrent aphthous ulceration)

2.1 Types and clinical features of recurrent oral ulceration

2.1.1 Minor aphthous ulceration

Minor aphthous ulcers is the most common type occurred from one to five, shallow, round or oval which affect the non-keratinized areas of the oral mucosa and less than 1 cm in diameter. The ulcers heal without scarring within 7-10 days and tend to recur at 1 to 4 month intervals.

2.1.2 Major aphthous ulceration

Major aphthous ulcers are larger than minor aphthae and greater than 1 cm in diameter, they may occur at any sites in oral cavity and persist for several months. The ulcers extend deeper and heal with scarring.

2.1.3 Herpetiform ulceration

The ulcers are 1-2 mm across with multiple, small, pin-head size and occur on any part of the oral mucosa. As many as a hundred ulcers may be present and heal within 7-10 days with scarring.

2.2 Possible aetiology of recurrent oral ulceration

2.2.1 Genetic factor

A family history is found in 45 percent of patients involving HLA antigens.

2.2.2 Trauma

2.2.3 Emotional stress

2.2.4 Infections

It has been reported that *Streptococcus sanguis* is isolated preferentially from the ulcers and there are some evidences of cross reacting antigens between *Streptococcus sanguis* and oral mucosa these could be involved in the immunopathogenesis of recurrent oral ulceration (Soames, and Southam, 1985).

2.2.5 Gastrointestinal diseases

2.2.6 Haematological deficiencies

Deficiencies of vitamin B12, folate and iron have been reported in up to 20 percent of patients.

2.2.7 Hormonal factor

There is a relationship between aphthae and stressful luteal phase.

2.3 Treatment of recurrent oral ulceration

2.3.1 Corticosteroids

Triamcinolone ointment, hydrocortisone pellets, and betamethasone spray can reduce the painful inflammation by an antiinflammatory action.

2.3.2 Tetracycline mouth rinse

There are some reports shown that tetracycline rinse significantly reduce frequency and severity of aphthae.

2.3.3 Chlorhexidine mouth rinse

2.3.4 Topical salicylate preparations

Salicylates have an antiinflammatory action and also have local effects.

3. Lichen planus

Lichen planus is a common chronic inflammatory disease of skin and mucus membrane and characterized by white striations often forming lattice like patterns, lesions usually bilateral and symmetrical. Erythema of varying severity is often feature and in the erosive form of the disease, the mucosa becomes ulcerated. These ulcerated areas are frequently large, irregular in outline and the floor of the erosions is covered by an adherent serious exudate. The erosions are almost always painful and require treatment. The common sites of the ulcers are buccal mucosa, dorsum of tongue and gingival. (Cawson ,and Odell, 1998)

Topical application of potent antiinflammatory corticosteroids is usually effective and gives good response.

III Pharmaceutical Aspects of Mucoadhesive System

Bioadhesion is an interfacial phenomenon in which two materials, at least one of which is a biological are held together by mean of interfacial force for an extended peroid of time. It also refers to any bonds produced by contact between two surfaces. In the case of bioadhesive drug delivery systems, the term bioadhesion is typically used to describe the adhesion between polymers, either synthetic or natural, and biological tissue.

In the case of polymer attached to the mucus or mucosal surface of tissue, the term mucoadhesion is employed (Castellanos, Zia, and Rhodes, 1993; Duchene, Touchard and Peppas, 1988; Mathiowitz, Chickering, and Lehr, 1999; Gandhi, and Robinson 1988).

In the last decade, mucoadhesive drug delivery systems have received considerable attention because of their ability to resolve several problems of controlled delivery such as the ability to prolong the residence time of dosage form and permit localization in particular regions to enhance drug bioavailability due to the strong interaction between a polymer and the mucosal tissue. Moreover, they inhibit the metabolizing of enzymes in a localized area (Castellanos, Zia, and Rhodes, 1993).

In the most instances, the mucoadhesive polymer is in contact with a soft tissue. Thus, the tissue layer responsible for formation of the adhesive interface is mucus (Castellanas, Zia, and Rhodes, 1993).

1. Characteristics of mucus layer

The mucus is the continuous layer covering the mucosa. It is secreted by the globet cells lining the epithelia or by special exocrine glands with mucus cell acini.

The composition and thickness of the mucus layer vary widely depending on anatomical location, sex and state of health. In general, it can be as thick as 1 mm in human and consists mainly of water, which represents more than 95%, glycoprotein 0.5-5%, electrolyte 1%, free proteins 0.5-1% and lipids in low proportions (Castellanos, Zia, and Rhodes, 1993; Duchene, Touchard, and Peppar, 1988; Mathiowitz, 1999).

Glycoproteins are the main mucus components, responsible for its viscosity (produce its unique gel-like characteristics), adhesive and cohesive properties. Basically, glycoproteins consists of a protein core possessing attached oligosaccharide chains (Figure 3a). Glucidic chains contain an average of about 8-10 monosaccharide residue of five different types consisting of L-fucose (6-deoxy-L galactose), D-galactose, N-acetyl-D-glucosamine (2-acetamide-2-deoxy-D-glucose), N-acetyl-D-galactosamine (2-acetamide-2-deoxy-d-galactose) and sialic acid. In human, the only

important sialic acid is N-acetylneuramic acid (5-acetamide-3, 5-dideoxy D-glycero-D-galacto-nonulosonic acid). Amino acids are principally serine, threonine and proline. Linkages between the protein core are of the O-glucidic type, between N-acetylgalactosamine and serine or threonine. Many of the terminal residues in the oligosaccharide side chains are sialic acid, which has an axial carboxyl group with negative charge at pH greater than 2.8, making the protein an anionic polyelectrolyte. Sulphate residues contribute equally to this negative charge. The mucus gel structure is the consequence of the intermolecular association of glycoproteins in a polymeric network. Previously thought to be a tetramer (Figure 3b.), the polymer is now believed to be a terminally linked chain with numerous cross-linkings. It has been proposed that the entanged nature of mucus is the result from disulphide bonds (intrachain) and macromolecular associations are due to physical bonds stabilized by electrostatic 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 4). An appreciable proportion of the glycoprotein is not incorporated in the network, but is present as a soluble fraction, enhancing the viscosity of the interstitial fluid rather than conferring a solid/liquid character. (Castellanos, Zia, and Rhodes, 1993; Duechene, Touchard, and Peppas, 1998; Gandhi and Robison, 1988).

2. Mechanisms of bioadhesion

Logically, for bioadhesion to occur, a succession of phenomena, whose role depends on the nature of the bioadhesive, is required. The process involved in the formation of such bioadhesive bonds has been described in three steps. The first step involves intimate contact that 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 then takes place, or inter-penetration of bioadhesive chains with those of the mucus then takes place. Finally, chemical bonds can then settle (Castellanos, Zia, and Rhodes, 1993; Duchene, Touchard, and Peppas, 1988; Mathiowitz, Chickering, and Lehr, 1999).

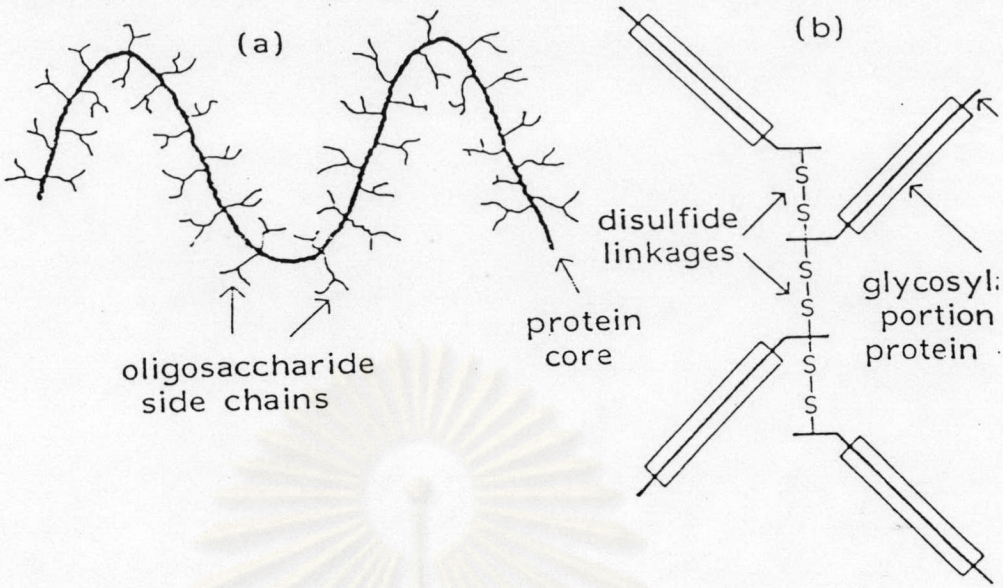


Figure 3 Schematic representtation of the mucus (a) glycoprotein (b) glycoprotein tetramer.

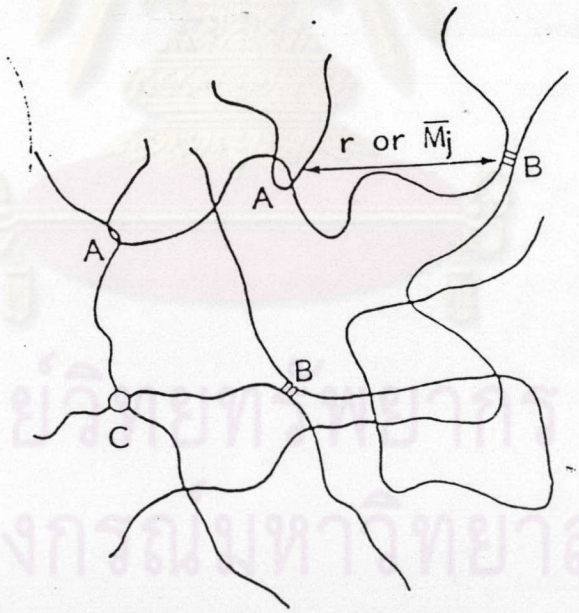


Figure 4: Crosslinked structure of the intestinal mucus network (a) entanglenents (b) molecular associations (c) permanent crosslink (r) average end –to– end distance between two junctions.

2.1 Intimate contact

The bioadhesive material has to penetrate the crevices of the tissue on which it is applied, and hence the tissue surface roughness is an important factor for bioadhesion. A rough surface or 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 5). Insignificant roughness for adhesive purposes occurs when the aspect ratio takes values of $d/h < 1/20$. For higher values of this ratio, only highly fluid materials can penetrate the tissue anomalies, and therefore their viscosity and wetting power are of the greatest importance for satisfactory bioadhesivity.

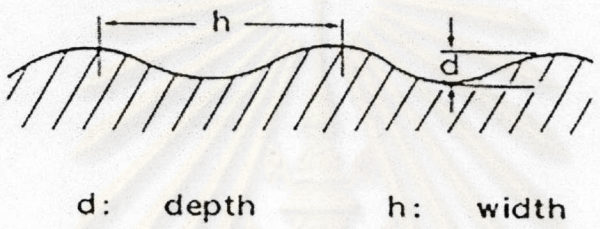


Figure 5 Surface roughness of soft tissue.

When the bioadhesive material is solid, its swelling in contact with moisture is necessary in order to impart sufficient freedom to the constituent chains (Duchene, Touchard, and Peppas, 1988).

2.2 Wetting and swelling

2.2.1 Wetting

The spreading coefficient S of a bioadhesive (Subscript b) with the tissue substrate (t) in the gastric area (g), for example, under static conditions, is given by the following equation:

$$S_{b/t} = \gamma_{gt} - \gamma_{bt} - \gamma_{bg}$$

where :

γ_{gt} = Interfacial tension between gastric content and tissue,

γ_{bt} = Interfacial tension between bioadhesive and tissue,

γ_{bg} = Interfacial tension between bioadhesive and gastric content.

For a bioadhesive material to displace the gastric contents and adhere spontaneously on the tissue, the spreading coefficient must be positive (Duchene, Touchard, and Peppas, 1988).

2.2.2 Swelling

The role of water in the mechanism of bioadhesion, for a solid material, is of primordial importance, as shown by Chen and Cyr (1970), who found that an optimum water content existed for maximum bioadhesion. Indeed, hydration of a colloid results in the relaxation of stretched, entangled or twisted molecules, which are able to liberate their adhesive sites giving them the possibility of creating bonds. It seems that the hydration of hydrocolloids causes dissociation of the already existing hydrogen bonding of the polymer. The polymer/water interactions may overwhelm the corresponding polymer/polymer interactions, favoring chain inter-diffusion. Water molecules form a double layer shielding any possible functional group interactions. The rupture of any interchain and intrachain associations increases the mobility of the macromolecules and facilitates their penetration in the surface crevices.

2.2.3 Interpenetration or consolidation

Interpenetration of chains from the bioadhesive polymer and mucus to a depth sufficient to create semi-permanent bonds corresponds to the diffusion theory discussed by Voyutskii (1971).

During chain interpenetration, 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 gradient. With crosslinked polymers, interpenetration of large chains occurs with greater difficulty. However, smaller chains and chain ends may still contribute to interdiffusion. After interpenetration, bonding occurred by secondary interactions with mucus glycoprotern is formed (Figure 6).

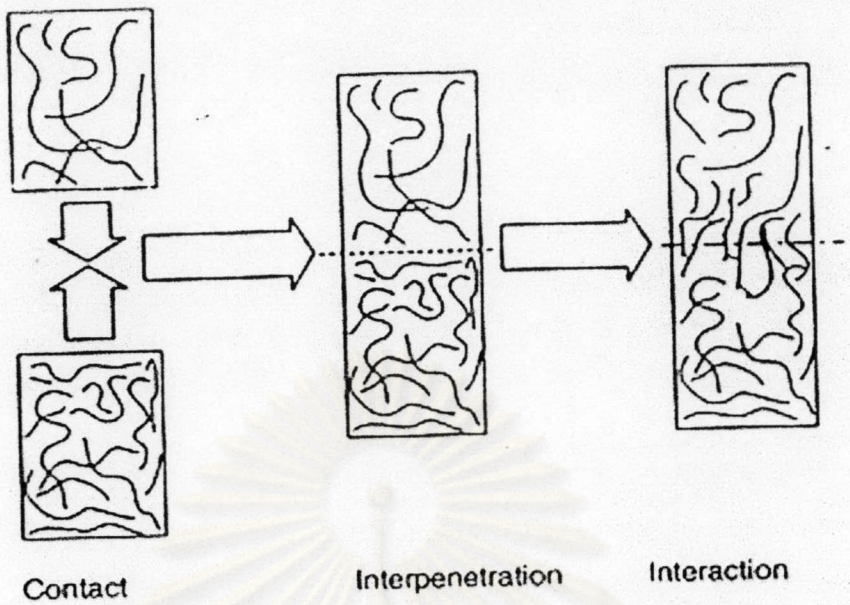


Figure 6 The interpenetration theory.

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

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

where :

e = interpenetration depth,

D_b = bioadhesive material diffusion coefficient through the mucus.

Evidence for this phenomenon is provided by an attenuated total reflection Fourier transform infrared (ATR-FTIR) studied by Jabbari et al. (1993). In their study, a thin cross-linked film of poly(acrylic acid) was formed on an ATR crystal. A mucin solution was placed into contact with this film and ATR-FTIR spectra collected over a period of time. Deconvolution of these spectra revealed a peak after 6 minutes at 1550 cm^{-1} (which manifested itself as a small shoulder in the original spectrum), which was attributed to mucin dimeric carboxylic C=O stretching (Figure 7) and it was proposed that this indicated the presence of interpenetrating mucin molecules within the poly (acrylic acid) film. The mucoadhesion bond occurs chiefly through chemical and physical interactions.

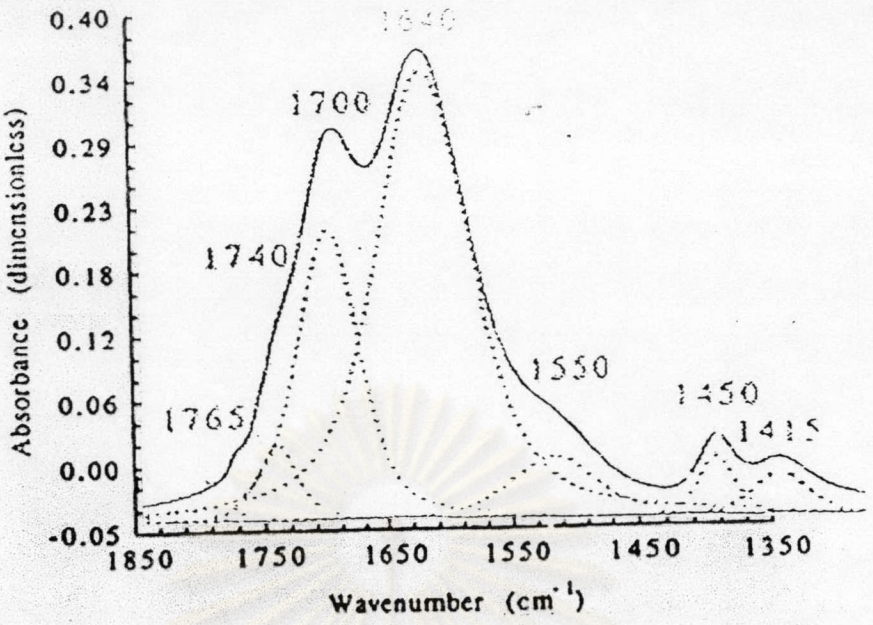


Figure7 The deconvolution of an ATR-FTIR spectrum of a cross - linked poly (acrylic acid) in contact with pH 7 buffered mucin solution(Jabbari et al.,1993).

3. Interaction

3.1 Chemical bonds

Type of adhesion chemical bonds include strong primary bonds which have a covalent nature, their high strength results in permanent bonds undesirable in bioadhesion. Secondary chemical bonds comprise a group of many different forces of attraction, including electrostatic forces (ionic bond) van der Waals forces, and hydrogen and hydrophobic bonds. Electrostatic attractions are due to Coulomb forces between molecules of opposite charge. Van der Waals forces are all the interactions between uncharged molecules. They can be attributed to three types of effect: 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 nonpolar molecules. Hydrogen bonding occurs when a specific hydrogen atom from one molecule is associated with another atom from a second molecule. Hydrophobic bonding occurs when nonpolar groups associate with each other in aqueous solution, due to the tendency of water molecules to exclude non-polar molecules.

Although system designed to form covalent bonds with proteins on the surface of epithelial cells may offer strength advantages, three factors may limit the usefulness of such permanent bonding. First, mucous barriers may inhibit direct contact of polymer and tissue. Second, permanent chemical bonds with the epithelium may not produce permanently retained delivery devices because most epithelial cells are exfoliated every 3 to 4 days. Third, biocompatibility of such binding has not been thoroughly investigated and could pose significant problems. For these reasons, many investigations have focused on developing hydrogel, mucoadhesive systems that bond through either van der Waals interactions or hydrogen bonds. Although individually these forces are very weak, strong adhesions can be produced through numerous interaction sites. Therefore, polymers with high molecular weights and high concentrations of reactive polar groups (such as –COOH and – OH) tend to develop intense mucoadhesive bonds (Durchene, Touchard and Peppas, 1988; Mathiowitz, Chickring and Lehr, 1999).

3.2 Physical or mechanical bonds

Mechanical bonds can be thought of as physical connections between surfaces similar to interlocking puzzle pieces. Macroscopically, they involve the inclusion of one substance in the cracks or crevices of another. On a microscopic scale, they can involve physical entanglement of mucin strands with flexible polymer chains and/or interpenetration into a porous polymer substrate. The rate of penertration of polymer strands into mucin layers is dependent on chain flexibility and diffusion coefficient of each. The strength of the adhesive bond is directly proportional to the depth of penetration of the polymer chains. Other factors that influence bond strength include the presence of water, the time or contact between the materials, and the length and flexibility of the polymer chains (Gandhi and Robinson, 1988; Mathionitz, Chickring and Lehr, 1999).

4. Theories of bioadhesion/mucoadhesion

Several theories of bioadhesion have been proposed to explain fundamental mechanism of attachment. In general, five theories have been adapted to the study of bioadhesion, the electronic, adsorption, wetting, diffusion and fracture theories. Some

are based on the formation of mechanical bonds, whereas others focus on chemical interactions.

4.1 The electronic theory

The hypothesis of the electronic theory relies on the assumption that the bioadhesive material and the target biological material have different electronic structures. On this assumption, when the two materials come in contact with each other, electron transfer occurs in an attempt to balance Fermi levels, causing the formation of a double layer of electrical charge at the bioadhesive-biologic material interface. The bioadhesive force is believed to be due to attractive forces across this electrical double layer. This system is analogous to a capacitor: the system is charged when the adhesive and substrate are in contact and discharged when they are separated. The electronic theory has produced some controversy regarding whether the electrostatic forces are an important cause of the result of the contact between the bioadhesive and the biological component (Gandhi and Robinson, 1988; Mathionitz, Chickring and Lehr, 1999).

4.2 The adsorption theory

The adsorption theory states that the bioadhesive bond formed between an adhesive substrate and tissue or mucosa is due to van der Waals interactions, hydrogen bonds, and related forces. Although these forces are individually weak, the sheer number of interactions can as a whole produce intense adhesive strength. The adsorption theory is the most widely accepted theory of adhesion (Gandhi and Robinson, 1988; Mathiowitz, Chickring and Lehr, 1999).

4.3 The wetting theory

The ability of bioadhesive or mucus to spread and develop intimate contact with its corresponding substrate is one important factor in bond formation. The wetting theory, which was developed predominantly in regard to liquid adhesives, uses interfacial tensions to predict spreading and in turn adhesion

Figure 8 represents a bioadhesive gel spreading over soft tissue in the gut. The contact angle (θ), which should be zero or near zero for proper spreading, is related to interfacial tensions γ through Young's equation:

$$\gamma_{tg} = \gamma_{bt} + \gamma_{bg} \cos \theta$$

where the subscripts t, g, and b stand for tissue, gastronintestinal (GI) contents, and bioadhesive polymer, respectively.

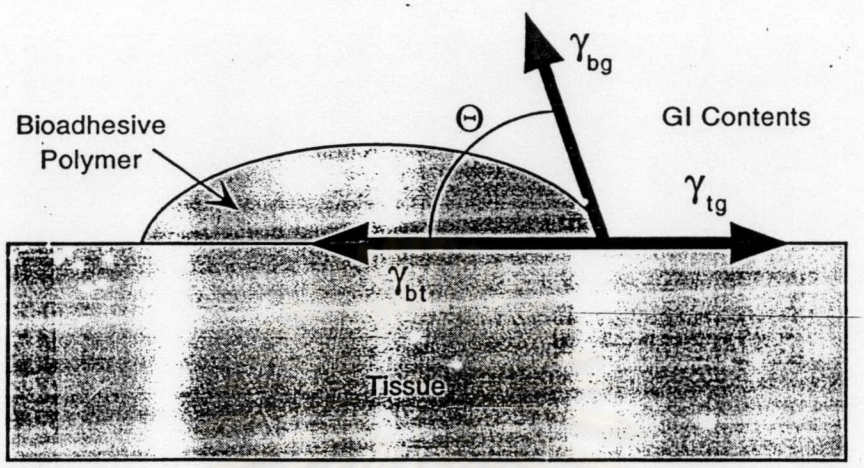


Figure 8 Schematic diagram showing the interfacial tensions involved in spreading a bioadhesive polymer over GI mucosa.

For spontaneous wetting to occur, θ must equal zero and, therefore, the following must apply.

$$\gamma_{tg} \geq \gamma_{bt} + \gamma_{bg}$$

The spreading coefficient, $S_{b/t}$ of a bioadhesive over biological tissue in vivo can be used to predict bioadhesion and can be determined as follows: (Castellanos, Zia, and Rhodes, 1993; Gandhi, and Robinson, 1988; Lehr et al., 1993)

$$S_{b/t} = \gamma_{gt} - (\gamma_{bt} + \gamma_{bg})$$

$S_{b/t}$ should be positive for a bioadhesive material that adhere to a biological membrane and the contact angle (θ) is obtaimed by the following equation :

$$\text{COS}\ \theta = (\gamma_{gt} - \gamma_{bt}) / \gamma_{bg}$$

Wachem et al. (1985, cited in Castellenos, Zia, and Rhodes, 1993) studied in vitro interaction of human endothelial cells with polymeric substances possessing different wettabilities in a culture medium containing serum. They found that moderately wettable polymers showed optimal adhesion, and that spreading and proliferation to cells and adhesion decreased or disappeared with either very hydrophilic or very hydrophobic polymers. In a homologous series of cellulosic polymers the authors observed an increase in bioadhesive strength as the contact angle increased.

On the other hand, for a solid material, the role of water in the bioadhesion mechanism is of primal importance, as shown by Chen, and Cyr (1970). The authors observed that maximum wet adhesive strength was attained when perfect matching of active adhesive sites was achieved in the presence of an optimum amount of water at or near the interface. If insufficient water was used to hydrate the dry hydrocolloid, active wet adhesion sites were not completely liberated and exposed for interaction. An excessive amount of water, on the other hand, caused over-extension of the hydrogen bonds and other adhesive forces leading to a weakening of the adhesive.

For the bioadhesive to displace GI luminal contents and make intimate contact with the biological tissue (i.e. spreading), the spreading coefficient must be positive. Therefore, it is advantageous to maximize the interfacial tension at the tissue-GI content interface (γ_{tg}) while minimizing the surface tensions at the other two interfaces (γ_{bt} and γ_{bg})

It is theoretically possible to determine each of the parameters that make up the spreading coefficient. The interfacial tension of the tissue-GI content interface (γ_{tg}) can be determined in vitro using classical Zisman analysis. The interfacial tension at the bioadhesive - GI contents interface (γ_{bg}) can be determined experimentally using traditional, surface tension-measuring techniques, such as the Wilhelmy plate method. Lastly, it has been shown that the bioadhesive - tissue interfacial tension (γ_{bt}) can be calculated as follows

$$\gamma_{bt} = \gamma_b + \gamma_t - 2F(\gamma_b \gamma_t)^{1/2}$$

where values of the interaction parameter (F) can be found in Wu (1970).

Extensive studies have been conducted to determine the surface tension parameters for several biological tissues (γ_t) and many commonly used biomaterials (γ_b).

The bioadhesive gel-tissue interfacial tension (γ_{bt}) has been shown to be proportional to the square root of the polymer-polymer Flory interaction parameter (c):

$$\gamma_{bt} \quad \propto \quad c^{1/2}$$

When c is small, the bioadhesive and biological components are similar structurally. This results in increased spreading and, therefore, greater adhesive bond strength.

Besides the spreading coefficient, another important parameter that may indicate the strength of an adhesive bond is the specific work of adhesion (W_{bt}). According to the Dupre equation, this is equal to the sum of the surface tensions of the bioadhesive and tissue, minus the interfacial tension .:

$$W_{bt} \quad = \quad \gamma_b + \gamma_t - \gamma_{bt}$$

Thus, using the wetting theory, it is possible to calculate spreading coefficients for various bioadhesives over biological tissues and predict the intensity of the bioadhesive bond. By measuring surface and interfacial tensions, it is possible to calculate work done in forming an adhesive bond. Both spreading coefficients and bioadhesive work directly influence the nature of the bioadhesive bond and therefore provide essential information for the development of bioadhesive drug delivery sysetms (Mathiowitz, Chickering, and Lehr, 1999).

4.4 The diffusion theory

The concept that interpenetration and entanglement of bioadhesive polymer chains and mucous polymer chains produce semipermanent adhesive bonds (Figure 9) is supported by the diffusion theory. It is believed that bond strength increases with the degree of penetration of the polymer chains into the mucous layer

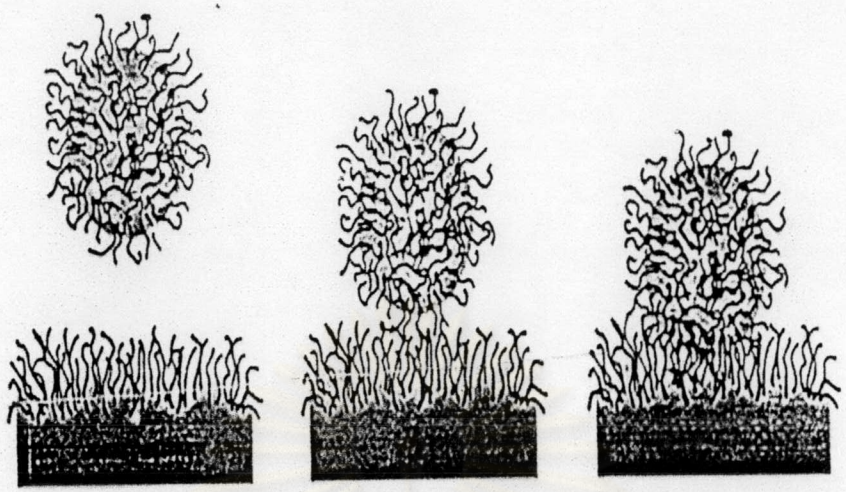


Figure 9 Mechanical bonding through interpenetration of bioadhesive and mucus polymer chains.

Penetration of polymer chains into the mucus network, and vice versa, is dependent on concentration gradients and diffusion coefficients. Obviously, any cross-linking of either component tends to hinder interpenetration, but small chains and chain ends can still become entangled. It has not been determined exactly how much interpenetration is required to produce an effective bioadhesive bond, but it is believed to be in the range of 0.2 – 0.5 μm. It is possible to estimate penetration depth (l) with the following.

$$l = (tD_b)^{1/2}$$

where t is time of contact and D_b is the diffusion coefficient of the bioad-hesive material in mucus. The bond strength for a given polymer is believed to be attained when the depth of penetration is approximately equal to the end-to-end distance of the polymer chains.

For diffusion to occur, it is important to have good solubility of one component in the other; the bioadhesive and mucus should be of similar chemical structure. Therefore, the strongest bioadhesive bonds should form between biomaterials whose solubility parameters are similar to those of the target mucus glycoproteins . Thus, the diffusion theory states that interpenetration and entanglement of polymer chains are

responsible for bioadhesion. The more structurally similar a bioadhesive is to its target, the greater the mucoadhesive bond will be (Gandhi and Robinson, 1988; Mathiowitz, Chickring, and Lehr, 1999).

5. The fracture theory

Perhaps the most applicable theory for studying bioadhesion through mechemical measurements has been the fracture theory. This theory analyzes the forces required to separate two surfaces after adhesion. The maximum tensile stress (S_m) produced during detachment can be determined by dividing the maximum force of detachment, F_m, by the total surface area (A_o) involved in the adhesive interaction:

$$S_m = F_m / A_o$$

In a uniform single-component system, fracture strength (S_f), which is equal to the maximum stress of detachment (S_m), is proportional to square root of fracture energy (g_c), Young's modulus of elasticity (E), and the critical crack length (C) of the fracture site, as described in the following relationship.

$$S_t \propto (g_c E/C)^{1/2}$$

Fracture energy (g_c) can be obtained from the sum of the reversible work of adhesion, W_r (i.e., the energy required to produce new fracture surfaces), and the irreversible work of adhesion, W_i (i.e., the work of plastic deformation at the tip of the growing crack), where both values are expressed per unit area of the fracture surface (A_f):

$$g_c = W_r + W_i$$

The elastic modulus of the system (E) is related to stress (σ) and strain (ϵ) through Hooke's law:

$$E = \left[\frac{\delta}{\varepsilon}\right]_{\varepsilon \to 0} = \left[\frac{F/A_o}{\Delta l/l_o}\right]_{\Delta l \to 0}$$

In this equation, stress is equal to the changing force (F) divided by the area (A_o), and strain is equal to the change in thickness (l) of the system divided by the original thickness (l_o).

One critical assumption in the preceding analysis is that the system being investigated is of known physical dimensions and composed of a single uniform bulk material. Considering this, the simple relationship obtained cannot be applied to analyze the fracture site of a multicomponent bioadhesive bond between a polymer microsphere and either mucus or mucosal tissue. For such analysis, the equations must be expanded to accommodate dimensions and elastic moduli of each component. Furthermore, to determine fracture properties of an adhesive union from separation experiments, failure of the adhesive bond must be assumed to occur at the bioadhesive interface. However, it has been demonstrated that fracture rarely if ever, occurs at the interface but instead occurs close to the interface.

Although these limitations exist, because the fracture theory deals only with analyzing the adhesive force required for separation, it does not assume or require entanglement, diffusion, or interpenetration of polymer chains. Therefore, it is appropriate for calculating fracture strengths of adhesive bonds involving rigid or semirigid bioadhesive materials, in which the polymer chains may not penetrate the mucous layer (Gandhi, and Robinson, 1988; Mathiowitz, Chickring ,and Lehr, 1999).

5. In vitro evaluation of bioadhesion / mucoadhesion

In order to assess the mucoadhesive drug delivery devices, rapid in vitro screening of material is desirable. Several suitable techniques have been reported. However, no attempt has been made to develop a standardized method of evaluation. Each technique has it own set of experimental conditions, and, therefore, it has been difficult to compare experimental findings among researchers. Some of the more significant methods that have been used for measuring and evaluating the interaction between bioadnesive polymers and biological substrates are as follows.

Kellaway (1990) classified these methods into mechanical and spectroscopic methods.

5.1. Mechanical methods

Mechanical methods are the commonly methods involve testing bioadhesive polymer against synthetic mucus, natural mucus, frozen or freshly tissue samples. This methods are based on the measurement of shear, peel and tensile stress. There have been two common methods consisted of the Wilhelmy plate method and shear test method.

The Wihelmy plate method, which is a modification of the Wihelmy was developed by Smart, Kellaway and Worthington in 1984, this method has been used for the measurement of superficial tension and involves a microbalance or tensiometer. The apparatus (Figure 10) consists of a glass plate coated with the polymer to be tested and immersed in a temperature controlled synthetic or natural mucus solution. The maximum force of detachment from the mucus, the surface tension and contact angle can be automatically measured using available software.

The shear test was investigated by Ishida, Numbu and Nagai (1983). The apparatus used for this experiment is shown in Figure 11. This method has been used for the measurement of the force required to shear another glass plate sliding on the ointment between two glass plates. The value of shear stress was read on the spring balance when the two glasses separated.

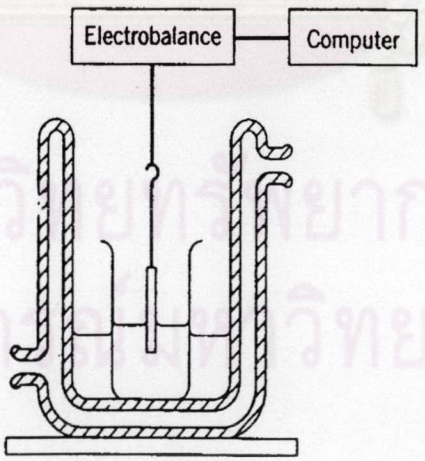


Figure 10 Diagrammatic of the Wilhelmy plate method (Smart et al. 1984 : 26).

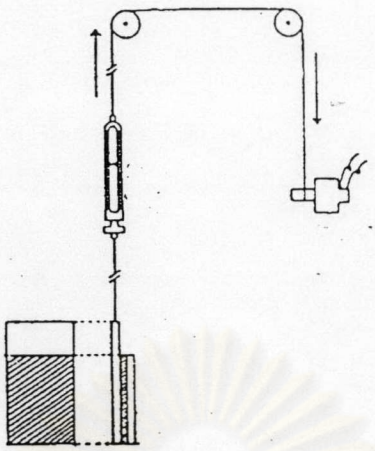


Figure 11 Schematic illustration of the shear test for mucoadhesion study (Ishida, Numbu and Nagai, 1983).

Another shear test method developed in 1998 by Rao, Vam, and Chary. The apparatus consists of two smooth plexi glass blocks, one was fixed on the leveled table. The tested polymer solution was kept on the centre of this block, the second block was place on and apply the weights to pull the upper block slide down from the base block. This weights will represent the shear stress of detachment. (Figure 12)

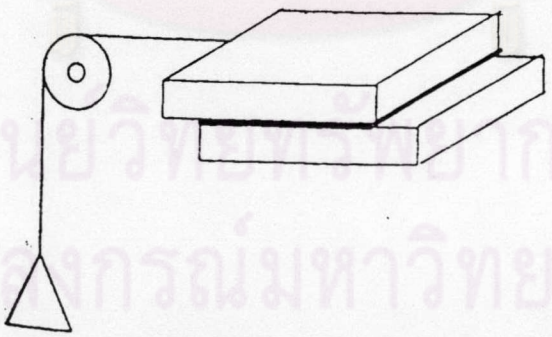


Figure 12 Shear stress measurement apparatus (Rao, Vani, and cherry, 1998).

A majority of other experiments has been variations of a simple tensile test that use either large tensile machine, modified tensiometers, or electrobalances.

In 1984, Gurny Meyer and Peppas developed a tensile method using an Instron tester equipped with a special cell for the determination of the adhesive bond strength (Figure 13). The cell is constructed of two plexiglass discs connected in their centres by permanently-fixed metallic bars perpendicular to the discs. The bars are fixed to the tensile tester. The two discs are enclosed in two cylindrical chambers. In the experiment, the bioadhesive preparation (gel) is hydrated with an equal amount of artificial saliva and placed between the two discs. The equipment is started and the discs are pulled at the constant rate, then the stress/strain curves are recorded.

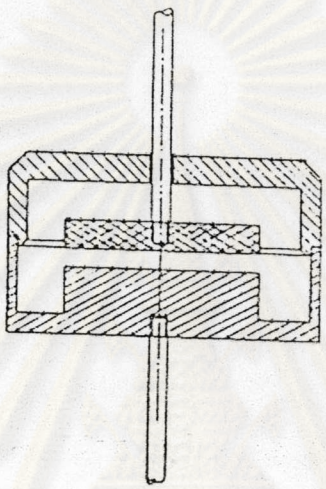


Figure 13 Schematic illustration of Gurny et al (1984) apparatus.

For the study of adhesion on biological tissue, there are a number of experiments use a tensile method for evaluating bioadhesion on biotogical tissue.

Ishida et. al (1981) was probably the first to describe an apparatus with the purpose of measuring the adhesiveness of an insulin solid dosage form for oral mucosa. The apparatus (Figure 14) requires the use of mouse peritoneal membrane on which the insulin dosage form is fixed and then wrenched with a spring balance.

In 1985 Ch'ng et al. uses the same type of system with rabbit stomach mucosa immersed in test solution (Figure 15). The mucosa is secured on a fixed holder (bottom) and on a mobile holder (top). This surface is coated with hydrated polymer, and raised in contact with the mucosa of the upper holder. After 1 minute of contact with a pressure due to the upper holder weight, the upper holder, connected to a

modified tensiometer, is raised with a force increasing at a constant rate of the polymer detaches from the mucus.

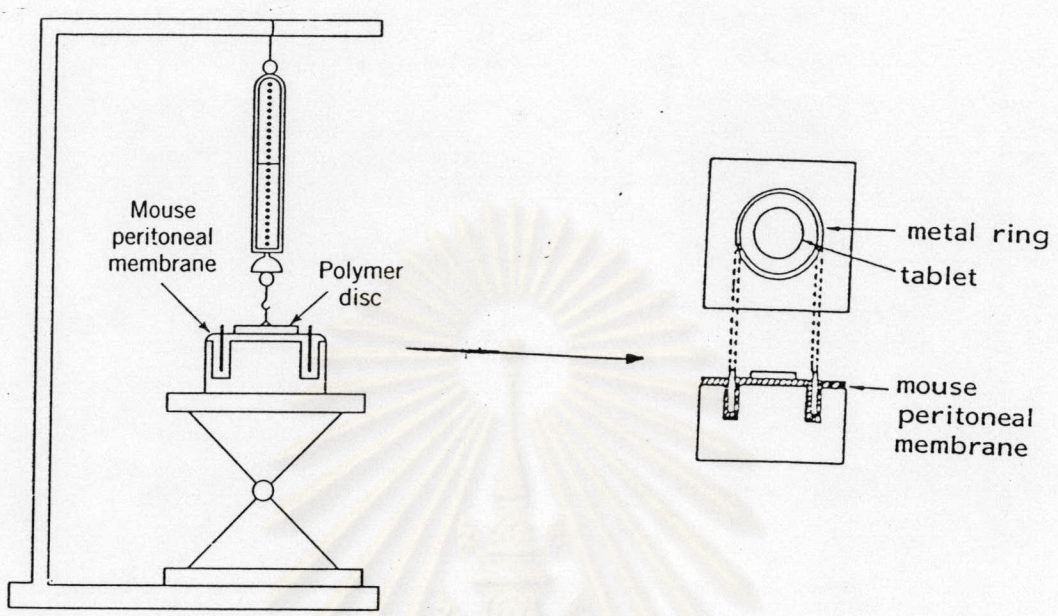


Figure 14 Diagrammatic representation of the adhesiveness measurement apparatus (Ishida et al., 1981 : 81).

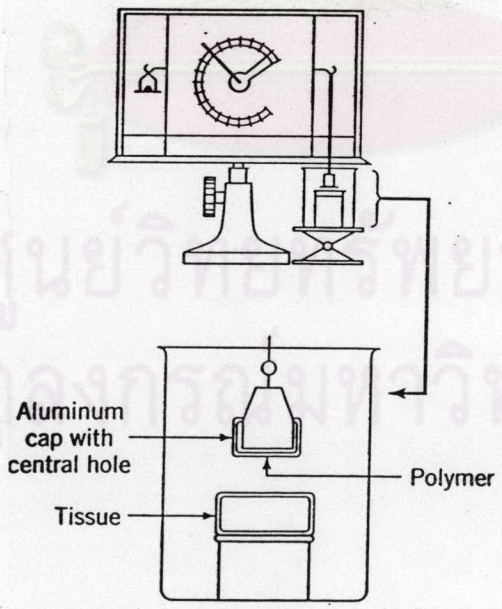


Figure 15 Diagrammatic illustration of the modified tensile strength test apparatus (Ch'ng et al., 1985 : 401).

In 1986, Ponchel et al. (Cited in Duchene, Touchard, and Peppas, 1988) developed the tensile apparatus use an Instron tester (Figure 16).

The mucosa employed is ox sublingual mucosa which is stuck on the holder connected to the lower clamp of the tester. A tablet made with the bioadhesive polymer is stuck on the holder connected to the upper clamp of the tester. The force necessary for detachment is continuously recorded, and enables the calculation of adhesion work.

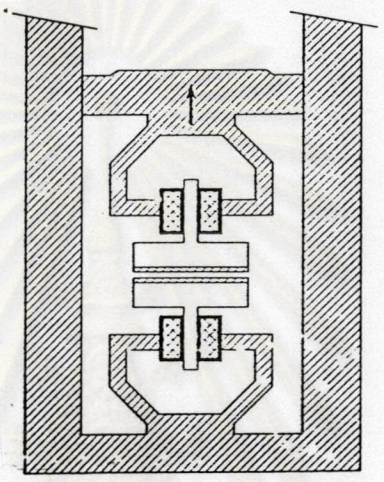


Figure 16 Schematic tensile tester apparatus (Ponchel et al., 1986).

An interesting method has been made to simulate the real behaviors of a gastrointestinal bioadhesive system on the mucus. The apparatus consists of a thin channel filled with a mucin solution, gel or natural mucus (Figure 17). The channel is thermostated and equipped with a transparent cover which can be removed by a handle. The system is connected through a valve to a fluid source which may be a gas or a viscoelastic liquid. The channel is placed on an optical microscope. For the experiment, a single spherical polymer particle of known weight is placed on the surface of the mucin using a Pasteur pipette, and the lid is closed. The volumetric flow rate (and the superficial velocity) of the fluid is then adjusted to physiological conditions, and the motion of the particle is followed with a fast-frame camera. The distance travelled by the particle is measured, as well as the time for detachment and the type of motion (rolling, sliding, jumping) (Dunchene, Touchard, and Peppas, 1988).

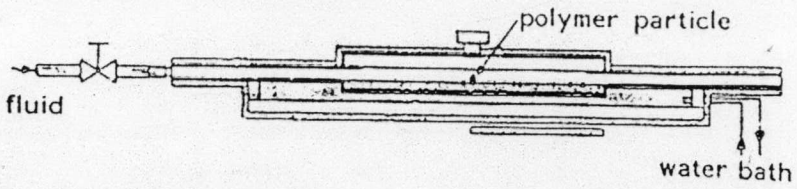


Figure 17 Schematic illustration of fluid flow chamber apparatus (Dunchene, Touchard, and Peppas, 1988).

Mortazavi and Smart (1994) developed an apparatus for measuring the duration of adhesion (Figure 18). The test disc was placed in contact with mucosal surface of isolated rat small intestine in an isotonic phosphate buffer chamber, then a constant tensile stress (loading weight) was applied and a timer mechanism activated. As soon as the adhesive joint failed this loading weight dropped on to a photocell which automatically stopped the timing device.

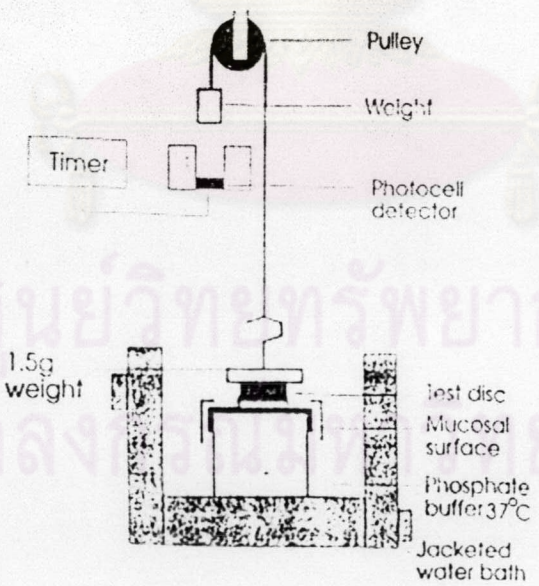


Figure 18 Schematic illustration of duration of adhesion apparatus (Mortazavi, and Smart, 1994 : 208).

A novel in vitro test was designed for measuring both bioadhesion. and the duration of adhesion (Gaserod et al., 1998). The apparatus used in this study is shown in Figure 19. Mucosal tissue was placed on the slope of aluminum plate then the test materials were deposited onto the tissue and synthetic mucus solution was pumped at the constant rate over the tissue, then the washed off materials were collected at different duration of time.

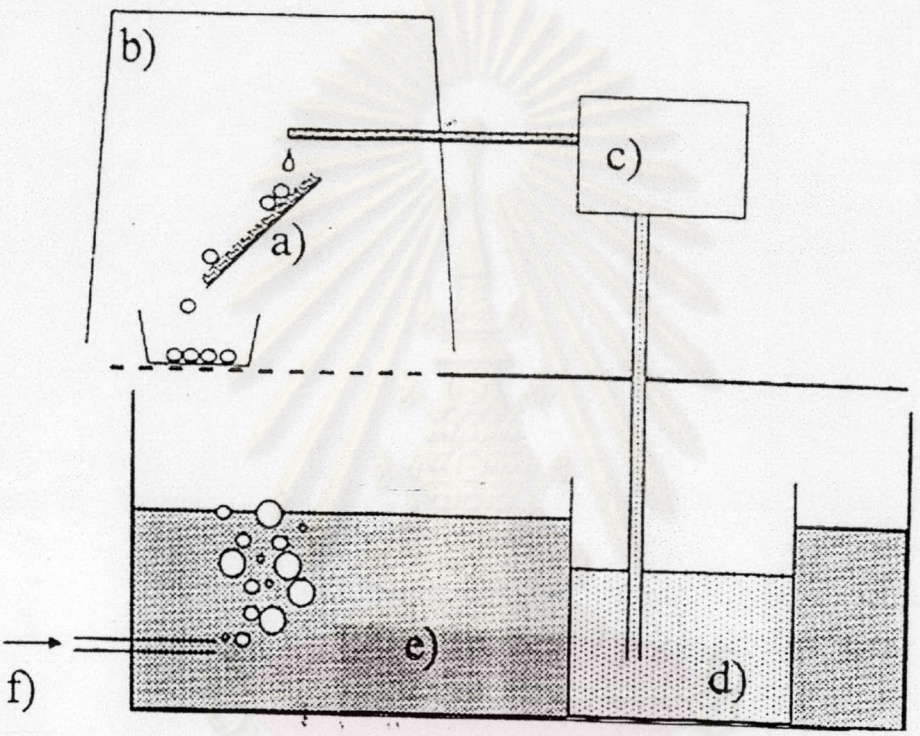


Figure 19 Schematic representation of the apparatus in the study of mucoadhesion of microsphere beads (Gaserod et al., 1998) (a) tissue, (b) humidity hood, (c) pump, (d) artificial saliva orgastric juice (e) water bathat 37 c, (f) airflow to promote humidification.

5.2. Spectroscopic methods

This methods include the fluorescent probe method, nuclear magnetic resonance and electron spin resonance experiments.

In 1984, Park and Robinson developed a fluorescence probe technique using cell cultures which indirectly measures the binding between a polymer and epithelial

cells. The binding of a polymer to a lipid bilayer of a cell membrane containing the fluorescent probe pyrene, which compresses the lipid bilayer, results in a change in florescence. The change in florescence is proportional to the degree of binding of the polymer to the cell membrane. This can be explained by the fact that photo-excited pyrene can react with non-excited monomer to form a complex called excimer. It is also possible to obtain information on cell polarity from the peak ratio measurement of monomer fluorescence, since the pyrene monomer is characterized by three well-defined peaks. Thus, the peak intensity ratio can be used as a measure of polarity of the probe environment.

Moreover, Qaqish and Amiji (1999) investigated the molecular mechanism of interactions between bioadhesive polymers and mucin by the fluorescence polarization method. Measurement of the percent change in polarization upon polymer-mucin association presented the mucoadhesion properties.

Mortazavi (1995) investigated interaction between mucoadhesive polymers and mucus by using C^{13}-NMR for examining the chemical shift of mucoadhesive polymers and evaluating the affect of hydrogen bond formation on the adhesive strength.

6. Factors influencing bioadhesion / mucoadhesion

The bioadhesive power of a polymer or of a series of polymers is affected by the nature of the polymer and also by the nature of the surrounding media. (Duchene, Touchard, and Peppas, 1998; Leung, and Robinson, 1990).

6.1 Polymer-related factors

6.1.1. Molecular weight

Gurny, Meyer, and Peppas (1984) reported that the bioadhesive force increased with the molecular weight of the bioadhesive polymer, up to 100,000, and beyond this level there was not much effect. It is clear that, to allow chain interpenetration, the polymer molecule must have an adequate length. It is also necessary to consider the size and configuration of the polymer molecule. Hence, for example, with polyethylene oxide, adhesive strength increases even up tp molecular weights of 4,000,000 : these polymers are well-known to contain molecules of highly linear

configuration, which contribute to interpenetration. On the other hand, with dextran, molecules with molecular weights as high as 19,500,000 do not exhibit better bioadhesion than molecules with a molecular weight of 200,000.

6.1.2. Concentration of active polymer

Bremecker (1983 Cited in Duchene, Touchard, and Peppas 1988) concluded that there was an optimum concentration of polymer corresponding to the best bioadhesion. In highly concentrated systems, the adhesive strength drops significantly. In fact, in concentrated solutions, the coiled molecules become solvent-poor, and the chains available for interpenetration are not numerous.

For solid dosage forms such as tablets, showed that the higher the polymer concentration, the stronger the bioadhesion (Ponchel et al., 1987).

6.1.3. Swelling

This characteristic is related to the polymer itself, and also to its environment. As mentioned earlier, interpenetration of chains is easier as polymer chains are disentangled and free of interaction. Swelling depends both on polymer concentration and on water presence. It must be remembered that, when swelling is too great, a decrease in bioadhesion occurs. Such a phenomenon must not occur too early, in order to lead to a sufficient action of the bioadhesive system. Nevertheless, its appearance allows easy detachment of the bioadhesive system after the discharge of the active ingredient. (Gurny, Meyer, and Peppas, 1984 cited in Duchene, Touchard, and Peppas, 1988).

6.1.4. Other physicochemical characters

Rathbone (1996) concluded that some physicochemical characters of mucoadhesive polymers which promote mucoadhesion should be as follows.

1) Generally hydrophilic molecules that contain numerous hydrogen bond forming groups.

2) Surface tension characteristics suitable for wetting mucus/mucosal tissue surfaces.

3) Sufficient flexibility to penetrate the mucus network or tissue crevices.

Furthermore, Park and Robinson (1984) investigated mucoadhesion by the fluorescence technique and summarized that cationic and anionic polymers bound with mucus more effectively than neutral polymers and degree of binding was proportional to the charge density on the polymer.

6.2 Environment-related factors

6.2.1. pH

The absorption of water by a polymer, and hence its swelling, depends to a great extent on the pH (Figure 20).

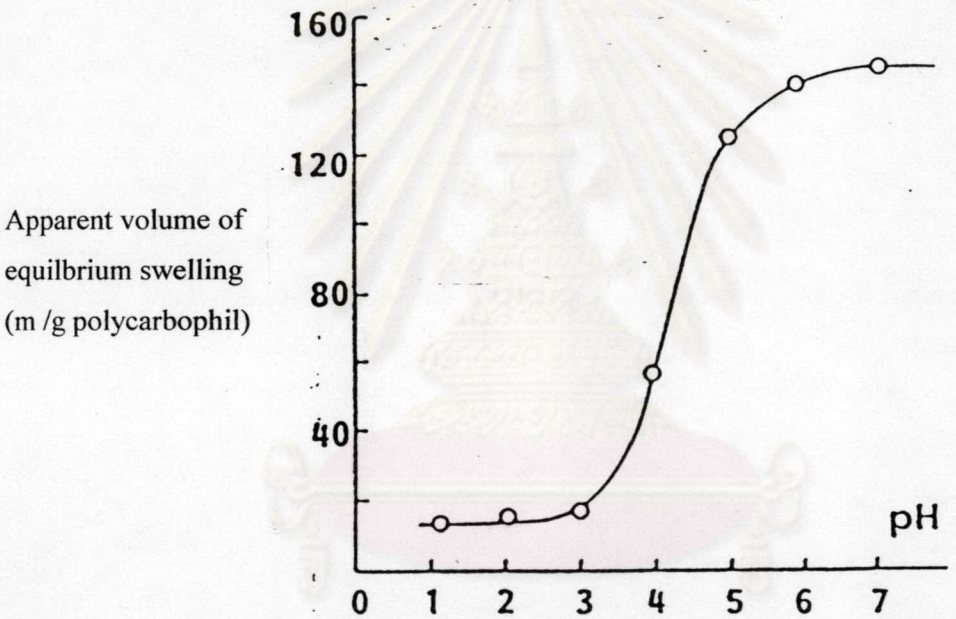


Figure 20 Apparent volume of equilibrium swelling of polycarbophil at various pH.

Bioadhesivity is also dependent on this factor (Figure 21) However, as can be seen when comparing the figures, when pH varies, polycarbophil swelling is not the dominant factor for bioadhesion. In any case, there is an optional pH for polymer adhesion (Ch'ng et al., 1985).

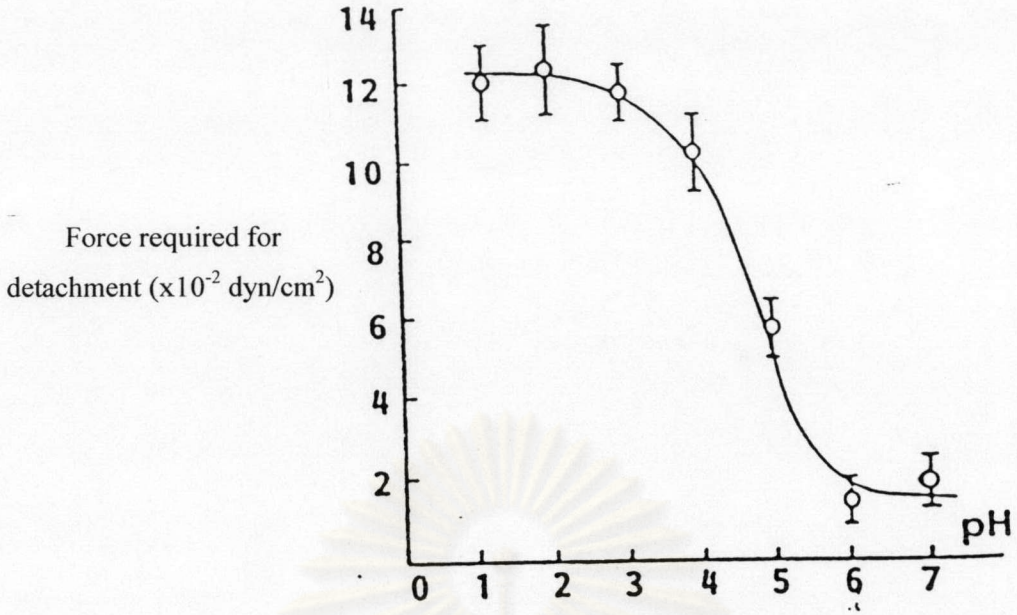


Figure 21 Effect of pH on in vitro bioadhesion of polycarbophil to rabbit stomach tissue.

6.2.2 Applied strength

It is obvious that, to place a solid bioadhesive system, it is necessary to apply a defined strength. Whatever the polymer, poly (acrylic acid/divinylbenzene), poly (HEMA) of Carbopol 934, the adhesion strength increases with the applied strength or with the duration of its application, up to an optimum (Figure 22) (Ponchel et al., 1987).

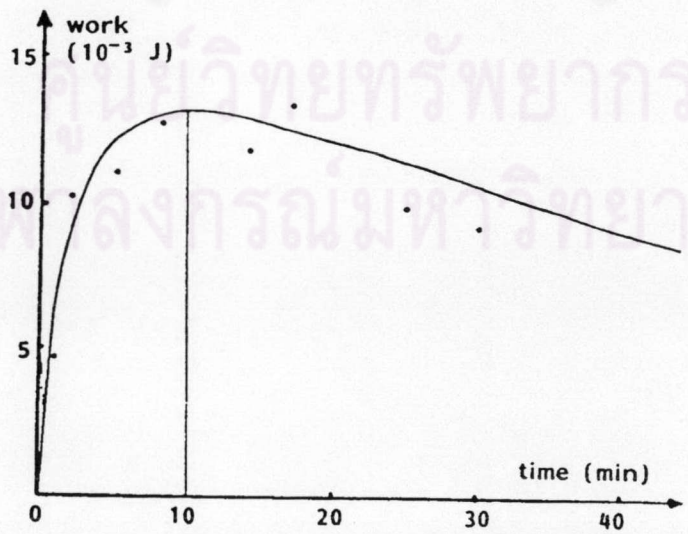


Figure 22 Effect of duration of applied strength on bioadhesion.

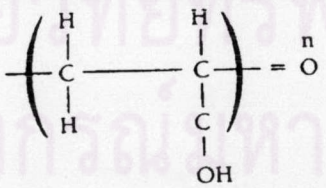
7. Biodhesive / mucoadhesive polymer

The first step in the development of buccal patch is the selection and characterization of an appropriate bioadhesive in the formulation. The polymers that are commonly used for the development of mucosal patches including chitosan, carbopol, cellulose derivatives (e.g., methylcellulose, sodium carboxymethy cellullose, hydroxypropyl methylcellulose hydroxyethylcellulose), natural gums (guar gum, Karaya gum, agarose), polyacrylates including poly (acrylic acid), poly (methacrylic acid), poly (vinylpyrrolidone), poly (ethyleneglycol) and gelatin. These polymers exhibit mucoadhesive properties in the presence of water (Lopez et al., 1998. Mathiowitz, 1999).

7.1 Carbomer

Carbomer is an anionic polymer which is widely used in mucoadhesive drug delivery system both alone and in combination with other polymers due to its excellent mucoadhesive performance (Ahuja, Dogra and Agarwal, 1995; Gupta, Garg and Khar, 1994; Ishida, Nambu and Nagai, 1983; Rao and Chary, 1998; San, Heuij and Tueker, 1992; Smart, 1991).

Its chemical name is carboxypolymethylene or carboxyvinyl polymer. It is a synthetic high molecular weight polymer of acrylic acid crosslinked with allylsucrose and containing 56-68% of carboxylic acid groups (Figure 26). It is soluble in water, alcohol and glycerin. Neutralized with alkali hydroxides or amines.



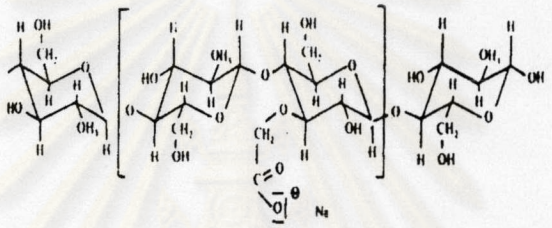
$$-\left(C_3H_4O_2\right)_x\left(-C_3H_5\text{-Sucrose}\right)_y-$$

Figure 23 Structural and empirical formulas of carbomer.

7.2 Sodium Carboxymethylcellulose (SCMC)

SCMC is an anionie polymer with strong mucoadhesive force which made it commonly used for mucoadhesive dosage forms (Jones, Woolfson, and Brown, 1997; San, Heuij, and Tukker, 1992; Smart, Kellaway, and Worthington, 1984).

SCMC is the sodium salt of polycarboxymethylether of cellulose (Figure 27). It is easily dispersed in water forming colloidal solution; pratically insoluble in alcohol, ether and most other organic solvents. The degree of polymerization affects the viscosity of solution. There are three viscosity grades; high, medium and low viscosities.



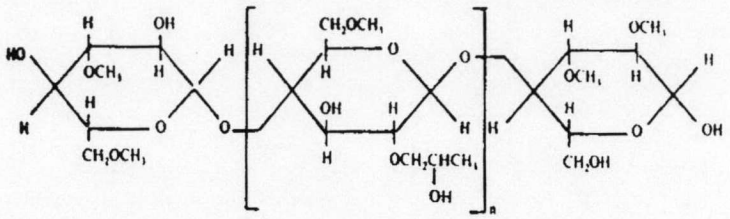
$$[C_6H_7O_2(OH)_{3-x}\ (OCH_2\text{-}COONa)_x]_n$$

Figure 24 Structural and empirical formulas of sodium carboxymethylcellulose.

7.3 Hydroxypropyl methylcellulose (HPMC)

HPMC is a nonionic polymer with good mucoadhesive performance. There are a number of reports revealed the application of HPMC in mucoadhesive preparations (Henriksen et al., 1996; Pyvik, and Graffner, 1992; Rodo, and Russell, 1988; Wong, Yuen, and Peh, 1999).

HPMC is a cellulosehydroxypropyl methylether (Figure 28). It is soluble in cold water, forming a viscous colloidal solution and undergoes reversible transformation from sol to gel on heating and cooling; insoluble in alcohol, ether and chloroform. Solutions of HPMC exhibit pseudoplastic rheology and stable at pH 3.0-11.0.



$C_8H_{15}O_6 - (C_{10}H_{18}O_6)n - C_8H_{15}O_5$

Figure 25 Structural and empirical formulas of hydroxypropyl methylcellulose.

7.4 Chitosan

Chitosan is a polycationic biopolymer consisting predominantly of long linear unbranched chains amino polysaccharide of β-(1→4)-2-amino-2-deoxy-D glucan or poly (N-deacetyl glucosamine) (Figure 29). It is obtained by alkaline deacetylation at high temperature of chitin, a polysaccharide which is the second most abundant in nature after cellulose. Chitin is a long linear chain of β(1→4)-2-acetamido-2-deoxy-D glucose or poly N-acetyl-D-glucosamine. It is a structural component of the exoskeleton of atropod, crustacean, marine invertibrate and insects (Bring, Sanford, and Zikakis, 1991; Felt, Buri, and Gurny, 1998).

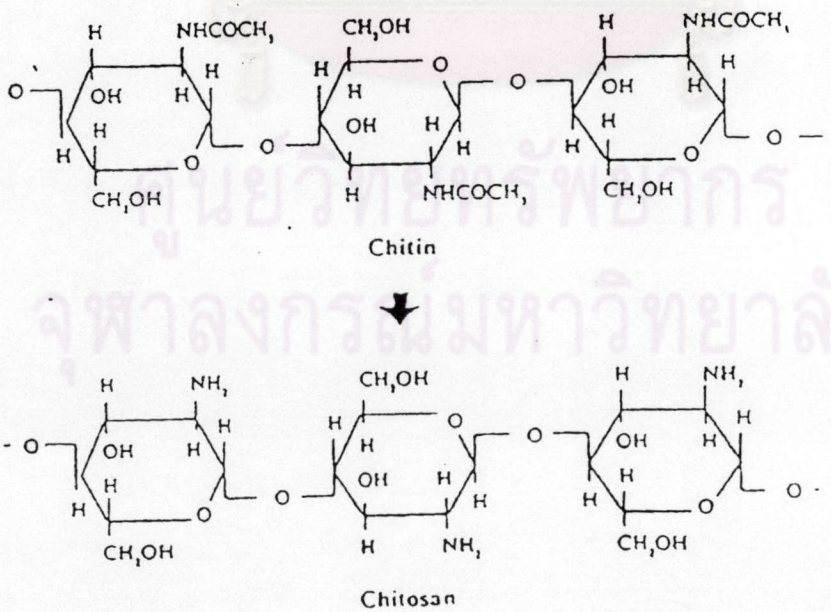


Figure 26 Structural of chitin and chitosan.

Chitosan is insoluble in water but soluble at pH values under 6.5 in most acidic media particularly organic acids such as formic, acetic, malic, tartaric, adipic and citric acid, where as acetic acid is commonly used as a reference.

On solubilization of chitosan, the amine groups in chitosan molecules are quarternized with acids. Chitosan is soluble in inorganic acids such as nitric acid or hydrochloric acid within the range of 0.15 to 1.1% acid by weight but it is insoluble in sulfuric acid (Mathiowitz, 1999; Skaugrud, 1989). It has attracted a great attention in the pharmaceutical and biomedical field due to its favorable biological properties such as nontoxicity, biocompatability and biodegradability. It is degraded by lysozymes into oligomers, which are further hydrolyzed to N-acctyl glucossamine (a common amino acid sugar in the body (Dumitriu, 1994; Mathiowitz, 1998). Moreover, Okamoto et al. (2001) investigated physical changes of chitosan in gastrointestinal tract and reported that chitosan can be degraded in the gastrointestinal tract.

Chitosan has been widely used in mucoadhesive drug delivery due to its excellent mucoadhesive properties over anionic and nonionic polymers (Lehr et al., 1992). There are a number of evidences revealed the interaction between chitosan and mucin, consisting of ionic bond hydrogen bond and hydrophobic interaction (Flebrig et al., 1995; Patel et al., 1999, 2000; Qagish, and Amiji, 1999; Rossi et al., 2000).

In addition, the ability of chitosan to form tough clear and flexible film, which is more stable at high humidity, leads the great attention to develop chitosan films for the application in controlled drug delivery and mucoadhesive drug delivery such as diazepam, nifedipine propanolol, glibenclamide, lidocain, amoxicillin, metronidazole and chlohexidine loaded chitosan films (Ilango et al., 1997; Kristl et al., 1993; Lehr et al., 1992; Lopez et al., 1998; Miyazaki, Yamaguchi, and Takada, 1990; Senel et al., 2000).

IV Buccal Mucoadhesive Dosage Forms

In general, buccal mucosa is more permeable than skin but less permeable than the sublingual that makes it a more suitable site for sustained release delivery. Most importantly, drugs administred via the buccal mucosa directly enter the systemic circulation there by avoiding enzymatic degradation in the gastrointestinal tract, as well as first-pass metabolism in the liver. Moreover, the administration of drug can be

stopped at any time by device removal and patient compliance is high due to the accessibility of the cheek lining and lack of invasive measures. Considerable attention is being focused on the buccal area for drug delivery purpose.

The mucoadhesive dosage forms for buccal route may be categorized into tablets, ointments, hydrogels and patches or films.

1. Tablets

There are a number of reports indicated that buccal mucoadhesive tablet can improve the mean residence time of the drug compared with intravenous formulations.

In 1981, Ishida et al. developed a new oral mucosal dosage form with a view to solving the problems of the administration of insulin, consisted of a core-base which contained cacao butter, insulin and additive, and a peripheral-base, which contained a mixture of hydroxypropyl cellulose-H (HPC) and carbopol934 (CP 934).

Schor et al. (1983) developed a nitroglycerin bioadhesive tablet, using a range of polymers made from naturally occurring materials (Synchron®) which can be mixed directly with an active pharmaceutical substance and directly compressed into tablets for the treatment of angina pectoris. The buccal tablet was quite small so that it would adhere to the buccal mucosa and not require adhesives to hold it in place.

Ishida, Nambu and Nagai (1982) also developed a bioadhesive tablet in order to produce a local anesthesia for toothache. This tablet (Figure 21) consists of a core containing the active ingredient, lidocaine, blended with a freeze-dried mixture of HPC and CP 934. The core is coated laterally and on the upper part with a bioadhesive mixture of HPC and CP, and directly compressed with a hydraulic press. Finally, a third layer is applied, consisting of a freez-dried mixture of HPC and CP added to magnesium stearate.

In 1985, Nagai and Machida developed a mucoadhesive bilayer tablet for aphtous stomatitis. The upper coloured layer consists of lactose and has no adhesive property, its role being to prevent active ingredient (triamcinolone acetonide) diffusion

out of its activity site (Aphtha®) and to allow an easy placing of the bioadhesive tablet. The lower layer, which contains the active ingredient, is made with HPC and CP 934, and constitutes the bioadhesive layer. This tablet commercially available under the name of 'Aftach'® (Figure 28).

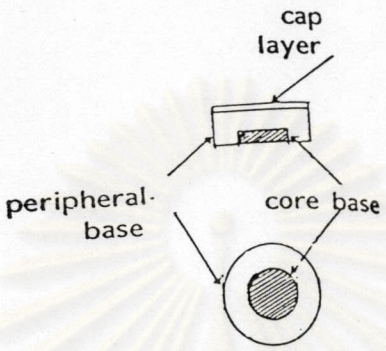


Figure 27 Lidocaine buccal mucoadhesive tablet.

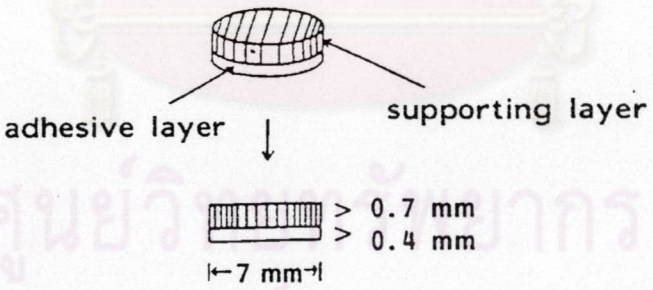


Figure 28 Aftach® buccal adhesive tablet.

Collins and Deasy (1990) developed two and three layered devices by filling the desired proportions of the components (cromadol, carbopol, cetylpyridinium chloride, flavors, HPC, magnesium stearate, precirol, spermaceti was and talc) of each layer into a punch and die set. After each layer was added, the fill was tapped down

and lightly compressed. The device offered considerable improvement over the proprietary product in sustaining salivary levels of drug in the therapeutic range.

Anlar et al.(1994) developed morphine buccal mucoadhesive tablet, which consists of hydroxypropyl methylcellulose (HPMC) and CP 910. The tablet was manually coated, on all sides except one to ensure unidirectional drug release.

In 1995, Ahuja, Dogra, and Agarwal reported that diltiazem could be formulated into polymeric buccal tablets. This multilayer tablets was comprised of a cap layer of CP 934 combined with either HPC, HPMC, or poly (vinylpyrrolidone) compressed using a die punch set around an already-compressed core of drug. (Duchene, Touchard, and Peppas, 1988).

Voorspoels, Comhaire and Remon (1997 Cited in Mathionitz, 1999) developed slow release degradable mucoadhesive tablets consisted of micronized progerterone), drum dried waxy maize starch, carbopol 974P and sodium stearylfumarate compressed into a tablet.

2. Ointments

In 1983, Ishida, Nambu and Nagai developed highly viscous ointment of Prednisolone for treatment aphthous ulcer using CP 934 as the mucoadhesive agent. The investigation showed that the release of prednisolone from an ointment-type oral mucosal dosage form containing 30% CP was better than the original ointment base.

Tretinoin mucoadhesive ointment (Bremecker, strempel and Klein, 1984), which consisted of neutralized polymethacrylic acid methyl ester, was formulated to treat lichen planus and reduce irritation to the mucus membrane.

3. Hydrogels

Hydrogels are characterized by hydrophillic polymeric networks that in the presence of water hydrate and swell.

In 1990, Warren, Kellaway and Timmins developed peptide hydrogel from CP 907 crosslinked with sucrose. To promote unidirectional drug delivery the mucoadhesive CP 907 film was backed by a less hydrophilic hydrogel. The backing membrane was composed of CP crosslinked with Caradol.

In 1993, Cassidy et al. investigated the transbuccal absorption of diclofenac sodium from a hydrogel formulated from 80:20 w/w hydroxyethyl methacrylate (HEMA) and a hydrophobic difunctional macromolecular crosslinker. The device however possessed no inherent mucoadhesive properties and was anchored to the mucosa of human volunteers by a rim of dental adhesive and covered with a backing of non-permeable Surlyn. A high loading of the drug within the hydrogel was achieved and the gel was applied in the hydrated state. It was found that the steady state flux of the drug across the buccal mucosa was 1000 fold greater than that observed across human skin in vitro. Cassidy, Landzert, and Quadros (1993) also developed the same hydrogel formulations of buprenorphine.

4. Patches or films

Mucoadhesive patches for administration to the mucosa of the oral cavity may have a number of different designs depending on various considerations, such as the therapeutic aim and the physicochemical and pharmacokinetic properties of the active ingredients. Regarding the therapeutic aim, two different rationales for developing mucosal patches may be differentiated: patches can be intended to deliver a drug to the systemic circulation in a way that is superior to other routes of administration or their purpose may be local therapy of the oral mucosa (Mathiowitz, Chickering, and Lehr, 1999).

The advantages of buccal patches are that the patches can be applied directly to the affected mucosal region have the potential to supply the site of action with effective drug levels and sustain these levels over a long period of time. Moreover, the affected mucosa is covered and protected from contact with food and from other mechanical stress that may cause pain and further irritation. Furthermore, patches

allow more exact dosing than the alternative gels or liquids. In addition, the patches have advantages over the buccal mucoadhesive tablets such as the patches are more convenient than the tablets which often difficult to maintain in suitable site in oral, to fail to swallow them and produce an uncomfortable feeling in the mouth. (Castellanos, Zia, and Rhodes, 1993; Mathiowitz, Chickering, and Lehr, 1999).

Patch size, geometry, and design depend not only on drug-related factors such as the dose, mucosal permeability, and physicochemical properties but also on considerations related to the acceptability of the product for patients.

Among the most important potential factors of patient acceptability are taste mucosal irritation, impediment of lip and cheek movement while speaking, eating, or drinking and an uncomfortable feeling in the mouth because of the continuous presence of a foreign body in the oral cavity. Frequently underestimated, the taste of many drugs is so bad that it can cause serious compliance problems if it is not either masked with appropriate additives or prevented from coming into contact with the taste receptors by the specific patch design, which is the more effective method (Rathbone ed, 1996).

A patch design for taste masking requires at least two layers. The mucoadhesive layer, which is applied to the mucosa, will in most cases also contain the active ingredient. The second layer functions as a backing layer covering the drug reservoir toward the lumen of the oral cavity; to be a good release barrier it has to be substantially less permeable to the drug and/or the saliva than the adhesive layer. For even more restricted access of saliva to the drug reservoir, the backing layer can be made larger than the drug-containing layer (Figure 29).

A patch with two or more layers, of which one of them being a backing layer, is also much more comfortable to wear than a single-layer patch. As the backing layer is not adhesive, it will not stick to the gums or teeth and thus will allow free lip and cheek movement around the jaws and teeth. Because of this, the adhesive layer can be formulated for much stronger mucoadhesion than with any one-layer design. Thus, non adherence and dosage form failure can be largely avoided, and patches can be developed for much longer application times. In addition, the impermeable backing layer has been considered to prevent drug loss from adhesive layer (Mathiowitz, Chickering, and Lehr, 1999).

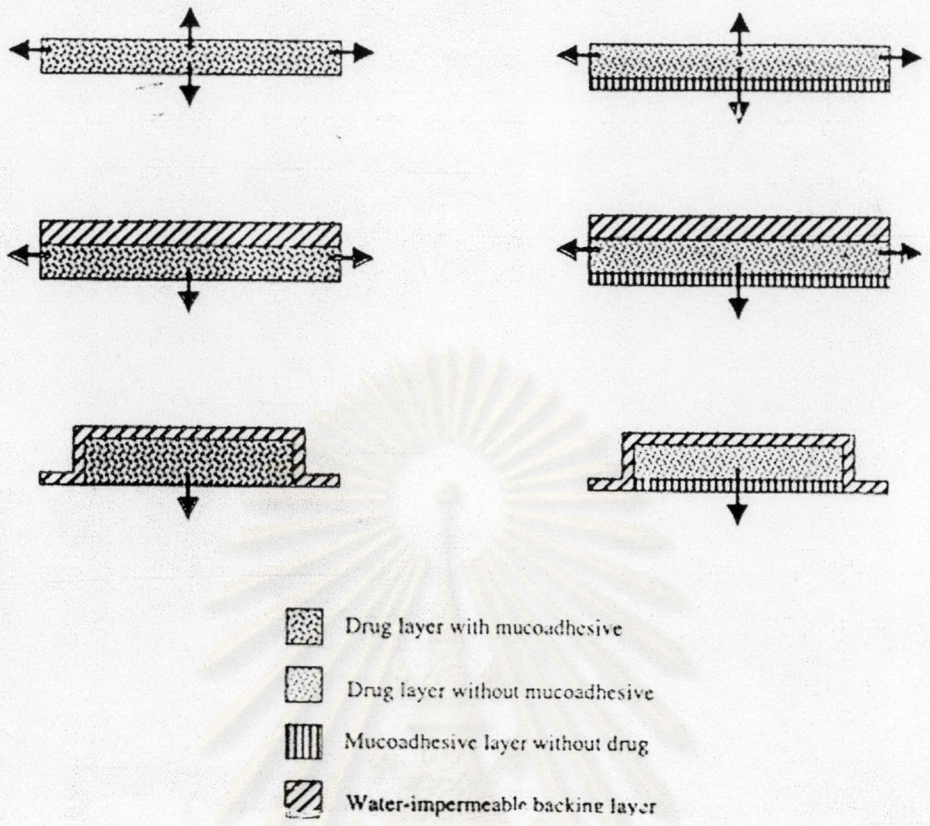


Figure 29 Schematic representation of some of the geometric patch designs.

Guo and Cooklock (1996) have studied the effects of backing layers on the swelling and adhesion of buccal patches. They found that ethylcellulose, a hydrophobic polymer, has very low water permeability and moderate flexibility; therefore, it is a good candidate for backing application. The effect of ethylcellulose on the hydration of polymer patches was significant, and the water uptake of polymer patches was delayed to about 24 hours by the application of ethylcellulose. The polymer patches that had a higher amount of ethylcellulose had a lower hydration rate. As alternative backing materials, polyvinylpyrrolidone and cellulose acetate mixture were also studied. The polyvinylpyrrolidone and cellulose acetate gel did swell with the polymer patch when the polymer patch was hydrated; however, this gel has very high water permeability and could allow the drug to pass through. Poly (ethylene-co-vinyl acetate) is another material that has been studied for backing layer application. Poly(ethylene-co-vinyl acetate) is a very hydrophobic and elastic polymer, and because most of the swelling force of the buccal patch was used to stretch the poly

(ethylene-co-vinyl acetate) film, the swelling ratio of the buccal patch significantly decreased when the patch was coated with poly (ethylene-co-vinyl acetate).

There are considerable studies involving buccal mucoadhesive Patches Yotsoyanagi, Yamamura, and Akao, (1985) designed a mucoadhesive using moderately water soluble polymer films containing analgesics and antibiotics for pain relief and aids in the healing of lesions. The film consisted of HPC containing tetracaine, thiamphenical and triacetin.

A patch of quite complex design was detailed by Veillard et al (1987), with unidirectional drug release assured by the presence of an impermeable backing membrane. The drug was incorporated into a rate limiting membrane sandwiched between the backing film and a mucoadhesive film prepared from polycarbophil. Details of the materials used in the fabrication of the patch were not published, however the device was observed to adhere to the mucosa of volunteers, with food and water permitted, for approximately 17 hours. The authors stated that the water swollen polycarbophil allowed some movement of the buccal membrane and thus provided comfort to the patient.

In 1988, Kurosaki et al (cited in Mathiowitz, Chickering, and Lehr, 1999) reported the use of a simple film of HPC for the delivery of propranolol. Rodu, Russell and Desmarais (1988) prepared a simple film by complexing HPC with tannic and boric acid (Zilactin®).

Anders and Merkle (1989) reported the fabrication of mucoadhesive two-ply laminate films. The backing layer, Multiphor sheet (commonly used as a backing layer for gel chromatography) faced into the oral cavity and was impermeable to the drug. An aqueous solution of a mucoadhesive hydrocolloid, hydroxyethyl cellulose (HEC), and the drug was cast into the sheet, and dried to form a laminate. The presence of an agarose graft on one surface of the Multiphor sheet ensured that the binding between the HEC and the backing film was stable even in the hydrated state. The devices, up to 12 cm^2 in area and loaded with protireline, were applied to the buccal mucosa of healthy volunteers. Adhesion to the buccal mucosa was observed for periods up to 30 minutes.

In 1992, Merkle and Wolany experimented with a number of polymers and different geometrie's for the design of patches for the delivery of different peptides, such as protireline and octreotide.

Guo (1994) developed buprenorphine buccal patch consisted of CP 934, polyisobutylene and polyisoprene as mucoadhesive polymers. The surface properties of the buccal patches were dependent upon the CP 934 content as well as the existing polymeric ratio. Polymer patches with higher CP content, or higher polyisobutylene : polyisoprene ratios; had higher water uptake capacity, which led to increased swelling and more buprenorphine release.

V Evaluation of Film

The evaluation tests designed to study the characteristics and final properties of free films are carried out by many utilizing laboratory techniques including thermal analysis, mechanical measurements, microscopic examination and diffusion experiments (Arvanito, Nakayama, and Aiba, 1998; Lim, and Wan, 1995; Nanthanid et al., 2001, Singh, and Ray, 1998). The most commonly used methods are thermal analysis and mechanical property evaluations. Other test procedures are considered on the basis of needful information in that investigation.

1. Thermal Analysis

Thermal properties of the membrane were examined by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and thermomechanical analysis (TMA). Thermogravimetric analysis is used to determine thermal stability of membranes and the upper limit of thermal stability is usually taken as the temperature at which weight loss of the sample begins. Differential scanning calorimetry is an extremely useful technique for measuring glass transition temperature (Tg) whereas thermomechanical analysis measures deformation of a substance under a non oscillatory load and can also conveniently measure transition from a glassy to a rubbery polymer.

2. Measurement of mechanical properties

Mechanical properties of a polymer are most conveniently determined by measuring their stress-strain relationship. Stress is the stretching force applied to the

sample, and the strain is the elongation of the sample under a given stress. Because the stress-strain relationship in membrane are time-dependent, the speed at which stress is applied is an important experimental parameter. Stress-strain measurements in polymers are usually performed on dumbell-shaped specimens as shown in Figure 30 .

To ascertain reproducibility of the test, care must be taken during cutting to avoid jagged edges. The specimen is clamped in a tester that is capable of extending the specimen at a chosen constant rate and measuring the force that the specimen exerts on a load cell. Percent elongation at break is also obtained from an examination. The tensile properties may vary with sample thickness, rate of grip separation, type of grips used and the method used to measure the extension. Generally, a suitable free film or membrane required adequate strength to avoid failure in use

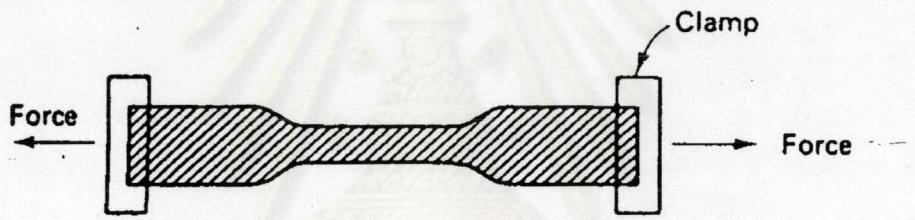


Figure 30 Typical shape of a flat polymer sample used for stress-strain tests.

A typical stress-strain curve for a thermoplastic material is shown in Figure 31. In the initial phase, application of stress causes a moderate elongation to the yield point, after which significant elongation takes place without greatly increased stress. Elongation then continues until the specimen breaks.

Characteristics of stress-strain curves are used to determine property of poly meric materials as shown in Figure 32 (Tsuruta et al., 1993).

3. Swelling property study

Degree of swelling and water sorption study are of considerable importance. When a film is placed in an aqueous environment, it will gradually absorb water, and the amount of absorbed water is determined by the polymer structure.

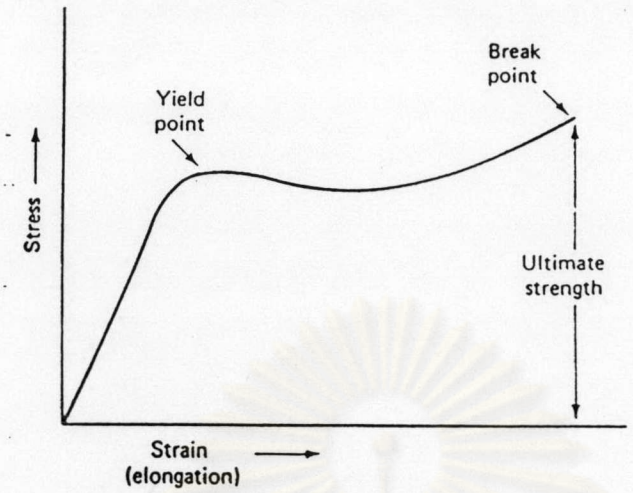


Figure 31 Stress-strain curve for a thermoplastic material.

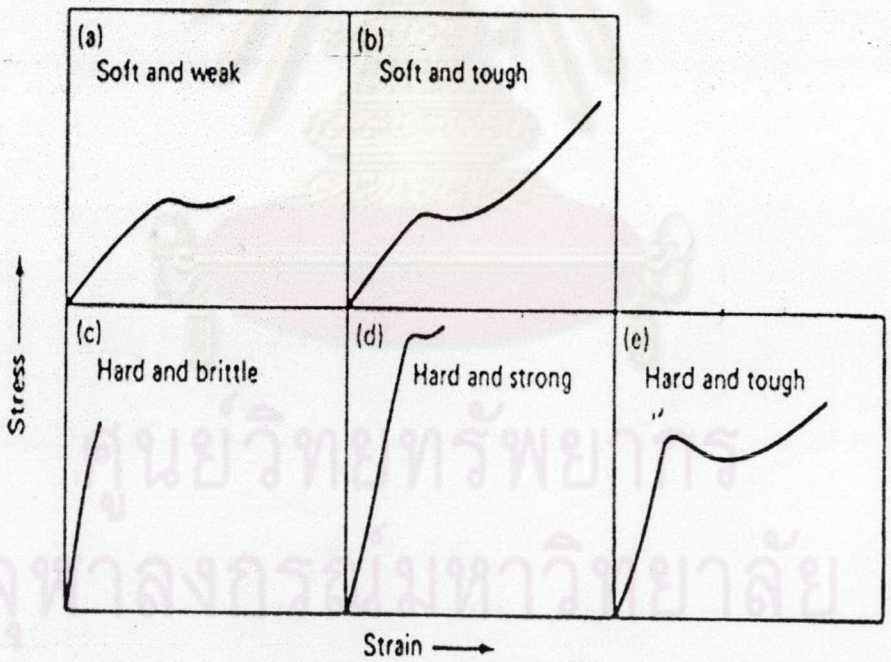


Figure32 Characteristic stress-strain curves for five different types of polymeric materials.

4. Surface characteristics and crystallinity

Scanning electron microscopy is the microscopic examination used to study surface characteristic and the homogeneity of a film. The film was mounted on a metal stub, coated with gold and examined using a scanning electron microscope.

X-ray diffraction is the method used to study crystallinity of a film. The film was mounted on a metal frame and scanned in suitable range of 2θ, and then determined the diffraction peaks obtained from X-ray diffractometer.

5. Drug Release from films

Diffusion test was a useful method to evaluate drug diffusion behavior and drug release rate from a film.

Mechanisms of drug release

5.1 Diffusion controlled release

In diffusion-controlled release systems, drug diffusion through the polymer is achieved by molecular diffusion due to concentration gradients. Depending on the molecular structure of the polymer, these systems may be classified as matrix (monolithic) and reservoir systems (Mathiowitz, 1999)

5.1.1 Matrix (monolithic) systems

In matrix (monolithic) systems, the bioactive agent is incorporated in the polymer phase either in dissolved or in dispersed form. Therefore, the solubility of the drug in the polymer becomes a controlling factor in the mathematical modeling of these systems. When the initial drug loading is below the solubility limit, release is achieved by simple molecular diffusion through the polymer. However, when the drug loading is above the solubility limit, dissolution of the drug in the polymer becomes the limiting factor in the release process. (Figure 33)

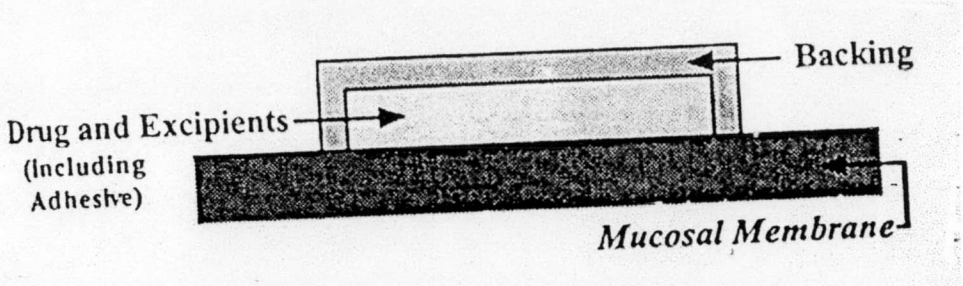


Figure 33 Illustrated matrix system.

When the bioactive agent is dispersed in the polymer phase, release kinetics known as Fickian release or Case I mechanism which have been derived by Higuchi (1963) by plotting the percentage of drug release as a function of the square root of time :

$$Q = kt^{1/2}$$

where Q is the amount of drug dissolved; t is the time and k is the liberation constant defined as :

$$k = \left[\frac{(D\varepsilon)}{T}(2A-\varepsilon Cs)\,Cs\right]^{1/2}$$

where D is the diffusion Coefficient; ε is the porosity factor of the matrix; τ is the tortuosity factor of the matrix; A is the amount of drug in the matrix (weight/volume); and C_s is the solubility of the drug.

In practice it is often found that the linear relationship between the amount of drug liberated and the square root of time is only true in part of the dissolution curve (60% of the time needed for complete liberation of the drug). Higuchi showed that in the matrix-type delivery system the porosity and degree of tortuosity in the capillaries influence the drug release rate. The amount of drug per unit of matrix volume decreases with the time as dissolution occurs) (Higuchi 1963; Tsuruta et al., 1993).

5.1.2 Reservoir systems

In a reservoir system the active agent is contained in a core that is surrounded by a rate-controlling membrane (Figure 34).

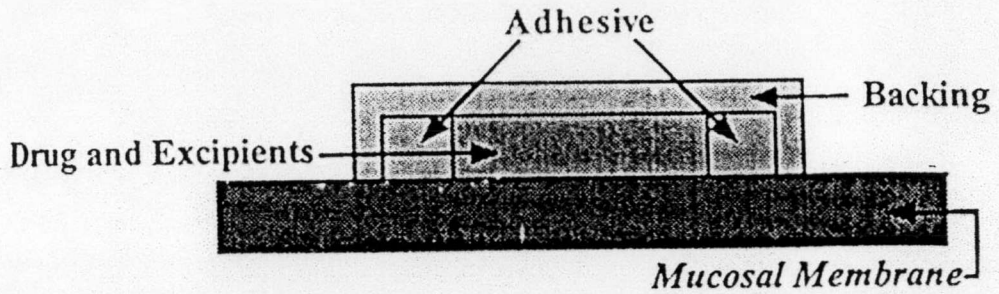


Figure 34 Illustrated reservoir system.

Transport of the material in the core through the surrounding nonporous, homogeneous polymer film occurs by dissolution at one interface of the membrane and then diffusion down a gradient in thermodynamic activity.

$$J = -D\frac{dC_m}{dx}$$

where J is the flux in g/cm^2sec, C_m is the concentration of the permeant in the membrane in g/cm^3, dC_m/dx is the concentration gradient, and D is the diffusion coefficient of the permeant in the membrane in cm^2sec.

At steady state the above equation can be rewritten as

$$J = \frac{DK\,\Delta C}{l}$$

Where ΔC is the difference in concentration between the solutions on either side of the membrane, K is the distribution coefficient and l is membrane thickness.

If the thermodynamic activity of the active agent in the reservoir remains constant, if there is no change in the rate-limiting membrane characteristics, and if infinite sink conditions are maintained at the downstream side of the membrane, rate of active agent release will be constant and can be predicted from a knowledge of membrane permeability and device configuration.

Thus, for a slab having a total surface area A, Fick's law can be restated as follows:

$$\frac{dQ}{dT} = \frac{A\,DK\Delta C}{l}$$

$$Q = \frac{A\,DK\Delta C.t}{l}$$

where Q is the amount of active agent released and $\frac{dQ}{dT}$ is the release rate at time t.

Drug release from this system is zero order, that is , the release rate of drug does not depend on time (Mathiowitz, 1999; Tsuruta et al., 1993)

5.2. Swelling controlled release

In swelling-controlled release systems, an active agent is homogeneously dispersed in a glassy polymer. Because glassy polymers are essentially impermeable, the active agent is immobilized in the matrix, and no diffusion through the solid polymer phase takes place.

When such a monolithic device is placed in an aqueous environment, water begins to penetrate the matrix and swelling takes place. As a consequence of the swelling process, chain relaxation takes place, and the incorporated active agent begins to diffuse from the swollen layer.

This process is represented schematically in Figure 35. One front separating the glassy from the rubbery state moves inward while a second front separating the swollen rubbery polymer from the surrounding aqueous environment moves outward.

In linear amorphous polymers, dissolution follows the swelling process, but crosslinked polymers or those containing significant chain entanglements, partial crystallinity will remain insoluble but will be mechanically weak (Tsuruta et al., 1993).

5.3 Erosion controlled release

The mechanism of drug release from soluble matrices is erosion. During dissolution, the outer gel layer fully hydrates, swells and erodes. The sequential steps of wetting, hydration, swelling and erosion continue throughout the dissolution course until the water-soluble components of the devices dissolve completely.

Mathematical expressions describing the release rates of solely erodible systems are usually involved and may be described only if the solubilization kinetics

of the polymers are known. This release mechanism is known as Super Case II and follows a super-linear kinetic of release as described by equation :

$$Q = kt^m$$

Where m is greater than l and depends on the relative rates of erosion and swelling of devices. In some cases, the release kinetics may approach zero order if the rate of solvent front advancing toward the device core and the rate of surface erosion are comparable and occur at the same time. Under such conditions, the drug diffusive pathlength will approximately remain constant and, assuming no surface area changes, provide zero order release kinetics. Since devices containing water-soluble polymers also swell and erode, it is possible to assume that the total surface area will remain constant for most of the dissolution course. (Tsuruta et al., 1993)

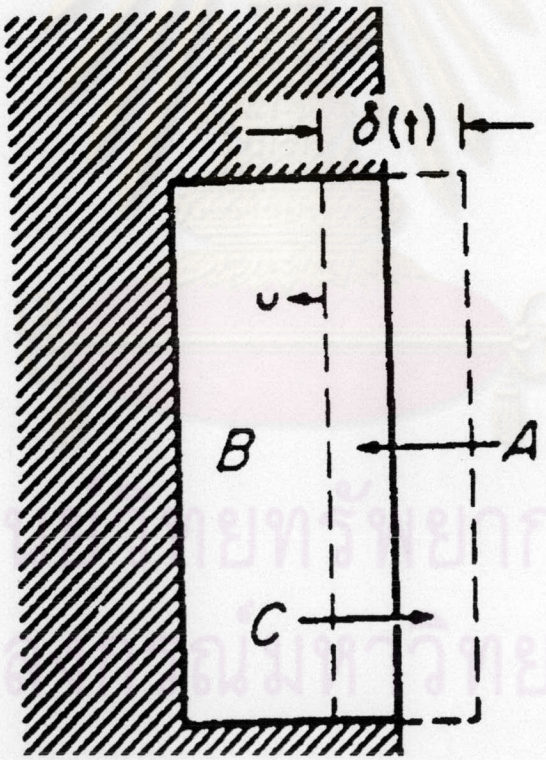


Figure 35 Schematic representation of swellingcontrolled release system. As the penetrant A enters the glassy polymer, B bioactive agent, C is released through the gel phase of thickness δ(t).

VI Stability assessment of the product

Although, there have been research works of *Garcinia mangostana* cream in laboratory scale and clinical studying, but stability assessment of this active ingredient in the product has never been reported. The stability test is performed under accelerated condition at 40°C, 75%relative humidity(RH) for three months. If amount of the active ingredients change not more than 10% of initial quantity, FDA justify granting a two years expiration period (Cartensen, 1990).