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Role of Polymer Physicochemical Properties on in vitro Mucoadhesion

Qing Zhang
University of the Pacific, q_zhang6@u.pacific.edu

ROLE OF POLYMER PHYSICOCHEMICAL PROPERTIES ON *IN VITRO* MUCOADHESION

By

Qing Zhang

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APPROVED BY

Thesis Advisor: Bhaskara R. Jasti, Ph.D.

Thesis Co-advisor: Xiaoling Li, Ph.D.

Committee Member: Ramakrishna Gadiraju, Ph.D.

Department Chair: William K. Chan, Ph.D.

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ROLE OF POLYMER PHYSICOCHEMICAL PROPERTIES ON *IN VITRO* MUCOADHESION

Abstract

By Qing Zhang

University of the Pacific
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Polymers with mucoadhesive properties are universally used in the development of mucoadhesive drug delivery system. Their physicochemical properties as well as the mechanisms related to their adhesive actions draw great attention for the modification of mucoadhesive properties.

In this study, relationships between physicochemical properties of hydroxypropyl methylcellulose (HPMC) compacts and mucoadhesive performance were investigated. Different commercial grades of HPMC (K3, E3, E5, E50, K4M, E4M and K15M) were prepared into compacts, and their surface hydrophilicity and hydration behavior were characterized. The *in vitro* mucoadhesive performance was determined by the tension strength between the compacts and different regions of mucous membrane (buccal, sublingual, stomach, and intestine). Positive correlations were found between: (1) viscosity of HPMC compacts and contact angle values measured by different simulated body fluids; (2) viscosity of HPMC compacts and *in vitro* mucoadhesive force; (3) contact angle values and *in vitro* mucoadhesive force. The hydration behavior exhibited improvement with the increasing viscosity of HPMC compacts. Moreover, the polar lipid content of each mucosa was likely an important factor affecting the mucoadhesion phenomenon.

Different ratios of ethyl cellulose (EC) was mixed with HPMC grade K15M to form combination compacts for the purpose of modifying the surface property. The mucoadhesive mechanism of both different grades of HPMC compacts and combination compacts were studied via the thermodynamic analysis of Lifshiz-van der Waals interaction and Lewis acid-base interaction. The total free energy of adhesion (ΔG^{TOT}) provided a prediction of an overall tendency of mucoadhesion, however, the results were showing disagreement with the measured mucoadhesive force. In general, the involving of EC in the combination compacts did not give a boost to the whole mucoadhesive performance.

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CHAPTER 1: INTRODUCTION

Drug absorption always has a limitation due to the short residence time of drug at the site of absorption [1]. For example, for ocular drug delivery systems, instinctive behaviors such as tear drainage and blinking can wash away the drug rapidly; for oral drug administration, drug and dosage forms are experiencing highly variable residence times at different sites in the gastrointestinal tract. Strategies such as mucoadhesion gained interests due to its ability of prolonging the mucosal residence time of drug delivery systems. In pharmaceutical sciences, mucoadhesion is defined as the state in which a material and mucus or a mucous membrane are held together for extended period of time by interfacial forces [2]. Mucoadhesive drug delivery system can induce high local drug concentration by retaining an intimate contact at the absorption site. Furthermore, the intimate and prolonged contact provides an opportunity of increasing the permeability of drugs including peptides and proteins. In recent years, such delivery system has been developed for oral, buccal, nasal, rectal and vaginal routes for both systematically and locally applications [3].

Mucoadhesion phenomenon has shown many path-breaking advantages, which includes (1) prolonging the residence time of the dosage form, thus enhance the therapeutic efficacy of the drug; (2) abundant blood supply and good blood flow rates which result in rapid absorption of the drug and faster onset of action; (3) bypassing first pass metabolism and thus improving drug bioavailability; (4) avoiding drug degradation in acidic environment of gastrointestinal tract; (5) ease of drug administration; (6) improving patient compliance [4, 5].

The selection of mucoadhesive materials for drug delivery has been of interest for decades owing to its importance of affecting drug absorption and its efficacy. For an ideal

mucoadhesive material, it should adhere rapidly to the desired region of mucous membrane without any physicochemical changes, release the drug without interference, possess biocompatible and biodegradable properties, protect the drug from enzymes degradation, and enhance the penetration of the drug [6]. Numerous mucoadhesive dosage forms are reported, including tablets, films, gels, creams, ointments, viscous solutions, micro- and nanoparticulate suspensions, and sprays [7]. One of the earliest mucoadhesive products that launched into the market is Orahesive®, which is a vehicle that deliver drugs to oral mucosa. This development further lead Orabase® into the clinical trials in 1959, which is a blend of polymethylene/mineral oil base [8]. Till now, many mucoadhesive products have been commercialized, for example, Replens® and Zidoval® gels, which are both for vaginal therapies [7]; Pilogel® and NyoGel® are ocular products [9]; Bunavail® and Zuplenz® are both oral film products [10].

1.1. Fundamentals of Mucoadhesion

1.1.1. Mucus Layer and Mucin

An in-depth knowledge about physicochemical properties of mucus layer is required for designing a successful mucoadhesive drug formulation. The presence of a complex mucus barrier lining the mucosal epithelium of tissues is one of the major challenges for mucoadhesive drug delivery systems [11]. Mucus layer cover the mucosal epithelium, with constantly exposure to the surrounding environment across the human body. It acts as a protection barrier against foreign particles, allows the entry and exit of nutrients and wastes, and can be served as a lubricant. While conducting its functions, mucus is continuously produced, secreted, and finally digested, recycled, or discarded [12]. The composition of mucus includes primarily of water (~95%), mucin glycoproteins (~2–5% w/v), lipids, DNA, non-mucin proteins, and cell debris. With its complicated biochemical composition, the mucus forms a dense and viscoelastic gel-like

layer over the epithelial cells which can adsorb a wide range of molecules and particles, including drugs, and other potentially harmful substances such as pathogens, toxins, and pollutants [13].

Mucins are macromolecules with very high molecular weight (10–40 MDa) secreted by epithelial goblet cells and submucosal glands. The primary structure of mucins consists of a high number of repeated proline, serine, and threonine residues ("PTS" protein backbone), with heavily dense of O-linked oligosaccharides (glycans) (Figure 1.1) [14]. N-acetylgalactosamine, N-acetylglucosamine, galactose, sialic acid, and fucose and low amounts of mannose and sulfate are considered as the predominant residues of the oligosaccharide side-chains [15]. Mucins are arranged in a brush-like structure due to these densely grafted glycans [11]. In addition to the glycosylated regions of mucins, the cysteine-rich globular domains (non- glycosylated) are responsible for the assembly of mucins into a 3D network via hydrogen bond interactions, disulfide bridges, and hydrophobic interactions between mucin monomer [16, 17]. High sialic acid (pKa∼2.6) and sulfate content (pKa<1) located on the terminal part of the glycoprotein molecules results in a strongly net-negative surface charge [18], thus the mucus exhibits sensitivity to pH and ionic strength similar to anionic polyelectrolytes [19].

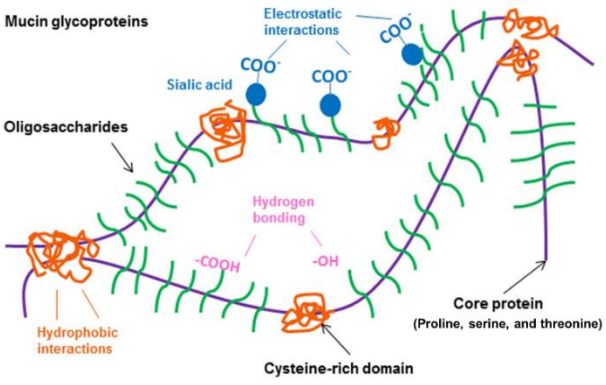


Figure 1.1. Schematic structure of mucin glycoproteins and their potentially mucoadhesive elements. Adapted from [20].

1.1.2. Mucus Physicochemical Properties

The permeability of drug and other molecules through mucus can be regulated by certain physicochemical characteristics such as pore size, viscoelasticity, pH, ionic strength and charge [11, 21]. The average mucus pore size was estimated range between 20 to 1800 nm among different organs and diseases [11]. It is reported that small molecules can freely diffuse through mucus, while macromolecules have limited penetration[12]. Furthermore, various studies demonstrated that decreasing particle size can result in increased mobility in the mucus [22, 23]. Thus, it is reasonable to deduce that the permeability of mucus can be limited by the pore size.

The viscoelasticity of mucus produces a balance between fluid-like (viscous) and solid-like (elastic) behaviors to maintain the normal physiological functions [17]. For example, a decrease in viscoelasticity promotes the growth of *Helicobacter pylori* infection in the gastric mucus [24], whereas hyper-viscous mucus is a major pathogenic feature of cystic fibrosis [25]. The pH of mucus shows variety in different organs of body [26]. The fluctuation of pH can alter the exposure of hydrophobic domains of mucins, the net charge of glycosylated domains of mucins, and non-covalent mucin–mucin interactions, and eventually induce the changing of the mucus conformation [21, 27, 28]. The ionic strength of mucus is modulated by epithelial channels and other cellular ion transport mechanisms [11]. It has a close relationship with the hydration state of mucus, and its osmolarity is isotonic in comparison with plasma [29]. A reduction in secreted electrolytes can result in dehydrated mucus as well as an increase in the mucus viscoelasticity [30, 31]. The ionic strength can also regulate the electrostatic interactions between the charged particles and the mucins [21]. In addition to these physicochemical properties, factors such as different body regions, pathological conditions, and individuals can also varying mucus permeability [13].

1.1.3. Mechanisms of Mucoadhesion

It is commonly described that the interaction between mucous membrane and mucoadhesive material takes place in two stages as shown in Figure 1.2:

- Contact stage: An intimate contact occurs between mucoadhesive material and mucous membrane.
- Consolidation stage: Physicochemical interactions occur to strengthen the adhesive joint, and further result in prolonged mucoadhesion.

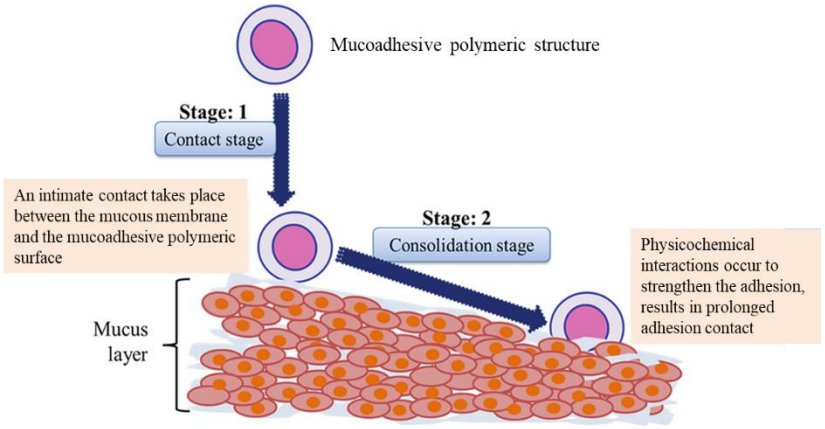


Figure 1.2. Scheme of the two-stages mechanism of mucoadhesion. Adapted from [32].

1.1.3.1. Contact stage. Contact stage is the initial step to form mucoadhesion, which is the intimate contact between mucoadhesive and mucous membrane. For oral region, corneal or vagina mucosa, the surface of mucoadhesive and mucous membrane can be physically brought together. In the nasal cavity or bronchi of the respiratory tract, the deposition of particles occurs by the inertial impaction process due to the aerodynamics of the anatomical site. However, the contact process has some difficulties in the gastrointestinal region (except oral and rectum). The adhesive material cannot be placed stably onto the target mucosal surface because of the gastrointestinal motility. Therefore, it is possible for uncontrollable and undesirable adhesion at unwanted locations to happen in such region [33].

When a mucoadhesive particle contact with a certain surface, two opposing forces may occur: attractive force and repulsive force. The attractive forces originate from van der Waal forces, surface energy effects and electrostatic interactions (if the surface and mucoadhesive particles possess opposite charges); while the repulsive forces also may include electrostatic interactions (if the surface and particles possess same charges). Other factors like osmotic pressure effects and steric effects can also affect the mucoadhesive process. The intrinsic property of the mucoadhesive particles, ambient environment and the distance between the particle and surface can lead to the net effect of these opposing forces. This contact process will be more complicated since mucus is gel layer instead of a simple solid. Thus, the mucoadhesive particle will be facing many challenges, such as overcoming the unstirred water layer adjacent to the surface or its physicochemical properties changes due to moisture and/or wrapped with biochemical molecules [33].

1.1.3.2. Consolidation stage. Consolidation stage is a very important process to combat the adhesive failure of the formulations to achieve stronger and prolonged mucoadhesion. There are two theories that are could explain this process. One of the theories is called diffusion theory, which indicates the mucoadhesive macromolecules will be relaxed by the presence of moisture and further interpenetrate with mucin glycoproteins by secondary interactions (predominantly van der Waal forces and hydrogen bonding). The other theory is dehydration theory, which states that after placing on a piece of mucous membrane, the mucoadhesive will rapidly dehydrate the mucus gel and consolidate the mucus joint until the equilibrium is reached.

The latter theory explains the quick nature of mucoadhesion, while the first theory requires the movement of macromolecules, which is a relatively slower process. However,

dehydration theory can only be suitable for dry or partially hydrated materials that are in contact with a sufficient amount of mucus gel and cannot be adapted to hydrated materials.

1.2. Theories of Mucoadhesion

Mucoadhesion is a complex process and six general theories have been proposed to explain this mechanism [34]. Due to the complexity of mucoadhesion, it is unlikely that a single theory could explain the mechanism completely.

1.2.1. Wetting Theory

The wetting theory is generally applied to mucoadhesive systems in the form of liquid or with low viscosity. It is associated with surface energy and interfacial energy between mucoadhesive and the biological substrate. It can be described as the ability of a liquid spreads out on a surface. Contact angle measurement is commonly used to evaluate the affinity between a liquid and a surface. Lower contact angle results in better affinity, thus the closer the contact angle to zero, the liquid flows and covers up more surface area and the maximum attractive forces can be achieved (Figure 1.4). The spreading coefficient can be defined by the surface energy parameters of liquid and solid as shown in the equation below [5]:

$$S_{AB} = \gamma_B - \gamma_A - \gamma_{AB} \quad (1.1)$$

Where γ_A is the surface tension (energy) of the liquid A, γ_B is the surface energy of the solid B and γ_{AB} is the interfacial energy between the solid and liquid. S_{AB} is required to be positive for the liquid to spread over the solid spontaneously.

When two unlike phases A and B are brought together reversibly, the work of adhesion (W_A) exists, which refers to the energy required to separate two phases and is given by:

$$W_A = \gamma_A + \gamma_B - \gamma_{AB} \quad (1.2)$$

The greater the individual surface energies of the solid and liquid relative to the interfacial energy, the greater the work of adhesion [35].

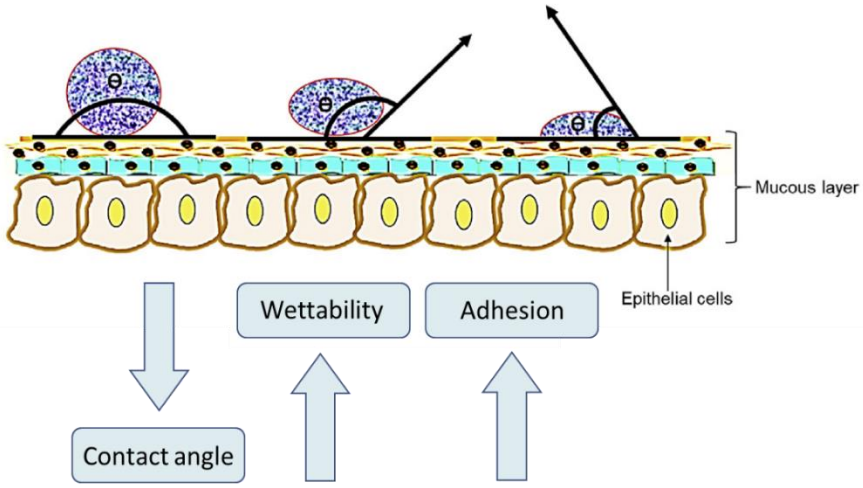


Figure 1.3. The relationship between the contact angle formed by the polymeric surface with the mucosal interface and the strength of adhesion. Adapted from [36].

1.2.2. Diffusion Theory

The diffusion theory describes the interdiffusion or interpenetration between mucoadhesive polymer chains and mucin glycoprotein chains with a sufficient depth to create a semi-permanent adhesive bond as depicted in Figure 1.4 [33]. A better mutual solubility between the mucoadhesive and the mucus results in stronger mucoadhesive bond [3]. The driving force for such a process is concentration gradient, the depth of interpenetration is dependent upon the time of contact and diffusion coefficients of both interacting substances. Many factors are involved in the diffusion process, the basic properties that will cause significant influences are molecular weight, cross-linking density, chain mobility/flexibility, expansion capacity of both networks and environmental-related factors [5]. It is documented that the depth of interdiffusion is required to reach 0.2–0.5 μm to generate an effective adhesive bond [3]. Another study showed that the polymer needs to have a critical chain length of at least 100 kDa

to form sufficient interpenetration and molecular entanglement [5]. The depth of interpenetration (L) between mucoadhesive polymer and mucin chains can be estimated by the following equation:

$$L = (tD_b)^{1/2} \quad (1.3)$$

Where t is the contact time; D_b is the diffusion coefficient of the mucoadhesive material in the mucus [3, 4].

1.2.3. Electronic Theory

This theory is based on the electronic differences between the structures. Mucous membrane is negatively charged due to the structure of mucin. Therefore, when the polymeric system and mucous membrane possess opposite electrical charges, and they come into contact, they will form an electronic bilayer at the interface, and the attractive forces between them will be enhanced (Figure 1.5) [5, 33].

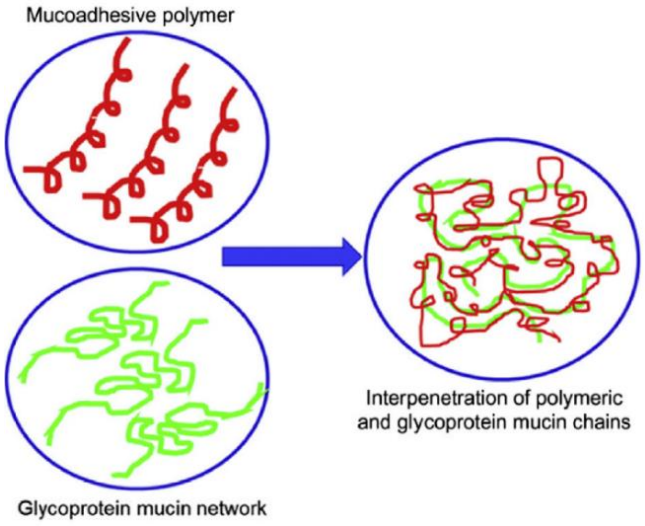


Figure 1.4. Diffusion interlocking model for the mucoadhesion of polymers. Adapted from [36].

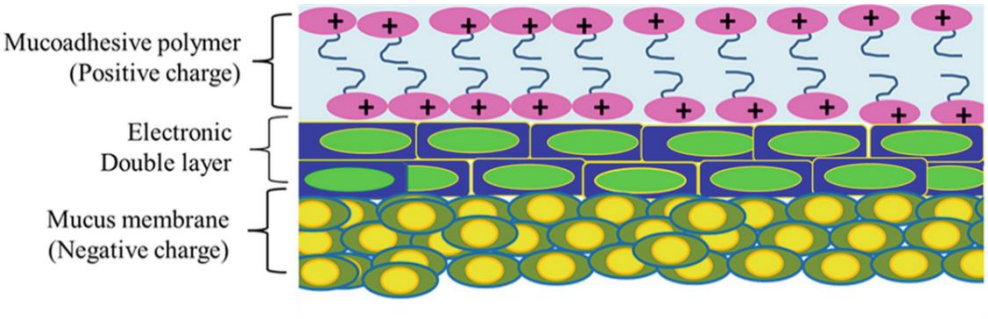


Figure 1.5. An electronic model of mucoadhesion. Adapted from [32].

1.2.4. Adsorption Theory

After an initial contact between two surfaces, the net result of primary or secondary forces give rise to the adhesion. The primary force of chemisorption provides a strong interaction across the interface resulting in ionic, covalent, or metallic bonding. Secondary forces are considered as the main contributors to mucoadhesive interaction, which mainly consist of van der Waals forces, hydrogen bonding, and hydrophobic effect [3].

1.2.5. Mechanical Theory

Mechanical theory proposes that an interlocked structure occurs by the penetration of adhesives into pores, cavities, and other surface irregularities on a rough surface thereby resulting in mucoadhesion [4].

1.2.6. Fracture Theory

The fracture theory examines the force involved in the separation of two adhered surfaces. This theory is commonly used in the evaluation of the mucoadhesion capacity in *in vitro* experiments. Theoretically, it assumes the fracture occurs at the interface between mucoadhesive and mucus. However, adhesive failure normally occurs at the weakest component, it is typical for the fracture to occur at one of the adhering surfaces, which is considered as a cohesive failure [35].

1.3. Factors Influencing Mucoadhesion

Generally, there are three factors influencing mucoadhesive performance, which include polymeric factor, environmental factor, and physiological factor [34].

1.3.1. Polymeric Factors

1.3.1.1. Molecular weight. It is understood that for a given polymer, an increase in molecular weight will lead to better mucoadhesive properties, it is also commonly accept that polymers with molecular mass ≥100 kDa have been found to have satisfied mucoadhesive performance in biomedical applications [3, 4, 36-38]. Interpenetration of polymer chains into mucus is more critical for low molecular weight polymers, while entanglement is more favored for high molecular weight polymers [34, 38, 39].

1.3.1.2. Hydrogen bonding capacity. Hydrogen bonding is one of the most crucial interactions that contributes to mucoadhesion. Polymers with hydrophilic function groups, such as hydroxyl or carboxyl groups, can induce hydrogen bonding between polymer and mucous membrane. The degree of hydrogen bonding depends on the structure of the polymer. The flexibility of the polymer is an important factor to improve the potential of hydrogen bonding by exposing more function groups to the mucous membrane [3, 34].

1.3.1.3. Hydration. Hydration is prerequisite for the expansion of mucoadhesive polymer and create mobility for the polymer chains to enhance the interpenetration between polymer and mucins. The swelling of polymer can improve the potential mucoadhesive performance by exposing more mucoadhesive sites for hydrogen bonding and/or electrostatic interaction between polymer and mucous membrane. However, the degree of hydration is required to be restricted due to excess hydration may cause adhesion failure and a slippery mucilage will form instead [3].

1.3.1.4. Degree of crosslinking. The density of crosslinking is inversely proportional to the degree of swelling [36]. With the increasing of crosslink density, the flexibility of the polymer chain will reduce, decreasing the swelling capacity of the polymer, limiting the interpenetration as well as entanglement, thus it will further cause weaker adhesion. However, a lightly crosslinked polymer is favored with sufficient hydration rate and flexibility which will facilitate its swelling degree [34].

1.3.1.5. Flexibility. In the consolidation stage, the diffusion of polymer chains into the interface region is critical. Therefore, it is important for polymer chains to contain sufficient flexibility to achieve the desirable entanglement and interpenetration with the mucus network. More structural flexibility of the polymer can lead to more exposure of the functional groups, which facilitate the formation of adhesive joint. Flexibility or mobility of the polymer chains is related to the viscosity, degree of crosslinking, hydration, and diffusion coefficient [5].

1.3.1.6. Charge. Anionic polymers exhibit excellent mucoadhesive properties owing to the formation of strong hydrogen bonding with mucus network. These polymers contain a great number of hydroxyls, carboxyl, and sulphate functional groups, inducing negative charges when the aqueous environment pH value is higher than its pKa value [40]. Chitosan is the one of the most studied cationic polymers with demonstrated superior mucoadhesive performance. As the pKa value of the amino groups on chitosan is around 6.5, it behaves as a polyelectrolyte with positive charge density at acidic and neutral pH [41]. Non-ionic polymers are also commonly used in the pharmaceutical formulations; however, they possess weaker mucoadhesive property in relative to polymers that carry charges.

1.3.1.7. Spatial conformation. The spatial conformation is required to take into consideration as some conformations may result in hindering the functional groups which are

responsible to form mucoadhesive bonds. Both helical structured polymer with high molecular weight and linear structured polymer with relatively lower molecule weight may produce similar mucoadhesive effect [42].

1.3.2. Environmental Factors

1.3.2.1. Applied strength and contact time. Either an increase of external applied strength or an extension of initial contact time can result in promoting mucoadhesive strength as well as the duration of mucoadhesion. If given a massive amount of applied strength for a long period of time, the polymer become mucoadhesive even if they do not have any attractive interactions with mucous membrane. The extension of initial contact time can lead to sufficient degree of swelling and increase the interpenetration depth between polymer chains and mucin chains [43].

1.3.2.2. pH. For anionic and cationic polymers, their mucoadhesive properties are relied on the functional groups which are ionized with charge distribution on polymer chains. The pH of the external environment determines the ionization of the functional groups. The charge density on the mucous membrane can be affected by the pH of the ambient environment. It can influence the dissociation of functional groups on mucin glycoprotein backbone [3].

1.3.3. Physiological Factors

1.3.3.1. Mucin turnover. The turnover of mucin molecules is a nature physiological clearance mechanism which can cause limitation of residence time for the mucoadhesive materials to stay on the mucous membrane. The detachment between mucoadhesive material and the surface will happen due to mucus turnover regardless of how strong the mucoadhesive strength. The mucin turnover varies in different physiological sites and in different individuals with a time range from few minutes to several hours [44, 45].

1.3.3.2. Disease state. The disease conditions will change the physicochemical properties of the mucous membrane, such as common cold, gastric ulcers, ulcerative colitis, cystic fibrosis, bacterial and fungal infections or inflammation. If the initial purpose of the mucoadhesive formulation is to apply on disease conditions, then the mucoadhesive property needs to be evaluated under the same conditions [45].

1.4. Mucoadhesive Polymers

In the development of mucoadhesive drug delivery system, a mucoadhesion promoting agent or polymer plays a significant role in the formulation due to its ability of increasing the residence time of the active pharmaceutical ingredient on a desired location. Polymers used in such system can be classified into many different categories based on the chosen criteria (Table 1.1). Brief details of some typical first or second generation mucoadhesive polymers are discussed in the following sections.

1.4.1. First-generation Mucoadhesive Polymers

The first-generation polymers are also known as traditional or non-specific polymers. Based on their carried charges, they can further be divided into three subsets: non-ionic polymers, anionic polymers, and cationic polymers [46].

1.4.1.1. Non-ionic polymer. Compare to the other two subsets, non-ionic polymers typically have weaker mucoadhesive properties. Polymers such as HPMC, MC, poloxamer, poly PVA and PVP are belong to this subset [5].

1.4.1.2. Anionic polymer. Anionic polymers are the most widely applied mucoadhesive polymers in pharmaceuticals due to their great mucoadhesive performance and minimum toxicity. As mentioned previously, these polymers contain a large number of hydroxyls, carboxyl and sulphate functional groups which lead to an overall negative charge at pH values

Table 1.1
Classification of Mucoadhesive Polymers [47]

Property used for classification	Categories	Examples
Generations	First-generation	Carbomer, polycarbophil, pectin, sodium alginate, Na CMC, CMC, Hydroxy ethylated starch, HPC, HPMC, PEG, PVA, PVP, Chitosan
	Second-generation	Lectins, bacterial invasins, thiolated polymers
Sources	Natural and modified natural polymers	Agarose, chitosan, gelatin, hyaluronic acid, carrageenan, pectin, sodium alginate
	Synthetic	*Cellulose derivatives* CMC, thiolated CMC, NaCMC, HEC, HPC, HPMC, MC, MHEC *Polymers based on poly(meth)acrylic acid* Carbomer, polycarbophil, polyacrylic acid, polyacrylates, copolymer of acrylic acid and PEG, copolymer of methyl vinyl ether and methacrylic acid *Others* Poly-N-2-hydroxypropylmethacrylamide, polyhydroxy ethylene, PVA, PVP, thiolated polymers
Aqueous solubility	Water soluble	*Cellulose derivatives* CMC, thiolated CMC, NaCMC, HEC, HPC, HPMC, MC, MHEC *Polymers based on poly(meth)acrylic acid* Carbomer, polycarbophil, polyacrylic acid, polyacrylates, copolymer of acrylic acid and PEG, copolymer of methyl vinyl ether and methacrylic acid *Others* Poly-N-2-hydroxypropylmethacrylamide, polyhydroxy ethylene, PVA, PVP, thiolated polymers
	Water insoluble	EC, polycarbophil

(Table 1.1 Continued)

Charge	Anionic	Carbomer, polycarbophil, pectin, sodium alginate, Na CMC, CMC
	Cationic	Amino dextran, dimethylaminoethyldextran, chitosan
	Non-ionic	Hydroxy ethylated starch, HPC, HPMC, PEG, PVA, PVP
Possible mechanism of formation of bioadhesive bonds	Covalent	Cyanoacrylate
	Hydrogen bonds	Acrylates, carbomer, polycarbophil, PVA
	Electrostatic interactions	Chitosan

Notes. Classification of polymers in examples are in italics.
CMC = carboxymethylcellulose; HPMC = hydroxypropyl methylcellulose; PEG = polyethylene glycol; PVA = polyvinyl alcohol; PVP = polyvinylpyrrolidone; HEC = hydroxyethyl cellulose; HPC = hydroxypropyl cellulose; MC = methylcellulose; MHEC = methyl hydroxyethyl cellulose; PAA = polyacrylic acid.

greater than the pKa of the polymer. Due to the negativity of the mucous membrane, a result of electrostatic repulsion will occur between the polymer and mucin, and further cause the uncoiling of polymer chains. This uncoiling process can improve the mechanical entanglement and interaction between polymer chains and the mucin glycoprotein [34]. Polymers with these functional groups can form strong hydrogen bonds with mucus network, so that they can exhibit outstanding mucoadhesive property. Typical examples of these polymers include PAA and its crosslinked derivatives, and NaCMC [5, 48].

Both polycarbophil and carbomer are PAA derivatives which have been studied widely as mucoadhesive platforms for drug delivery. Both compounds have the same acrylic backbone, the polycarbophil polymer is cross-linked with divinyl glycol, while the carbomer is cross-linked with allyl sucrose or allyl pentaerythritol. Polycarbophil has a very high swelling capacity under

neutral pH conditions, which allows greater interpenetration and entanglement within the mucous membrane [48]. Carbomer has a unique pH-triggered gelation behavior aside from its excellent mucoadhesive property, which gives a great opportunity of formulating into in-situ gelling dosage forms [49].

1.4.1.3. Cationic polymer. Among all the cationic polymers, chitosan is one of the most studied and abundantly used polymer in the pharmaceutical studies. Chitosan is considered as renewable, sustainable and affordable product, it is also a non-toxic material with great biocompatibility and biodegradability [50]. The primary amino groups on the structure of chitosan can bind with the sialic acid and sulphonic acid of mucin via ionic interactions. Additionally, the presence of hydroxyl and amino groups can interact with mucous membrane via hydrogen bonding. At acid environment (pH<6), the primary amino groups become protonated, and give rise to a net positive charge which can interact with negatively charged mucins by providing a strong electrostatic interaction. Aside from its mucoadhesive property, it also has film forming ability, antibacterial activity and wound healing properties, and is able to bind lipids and fatty acids due to its physicochemical and biological properties [38]. However, the water solubility of chitosan and its mucoadhesive performance is limited at neutral and alkaline pH values. Therefore, the chemical modifications of chitosan such as trimethyl chitosan, carboxymethyl chitosan, thiolated chitosan and others, are extensively studied [51].

1.4.2. Novel Second-generation Muco/bioadhesive Polymers

Due to the uncontrollable and targetless drawbacks of traditional first-generation mucoadhesive, scientists developed the second-generation muco/bioadhesive polymers. Other than mucous membrane, the second-generation mucoadhesive aims at a more accurate domain

such as the residues on the cell membrane, followed by an invasive mechanism accessing into the cell.

1.4.2.1. Lectins. Lectins can conduct an interaction of adhering to the cell surfaces, such process can also be referred as “cytoadhesion” [52]. Lectins are naturally occurring carbohydrate-binding proteins that bind reversibly with sugar groups of other molecules such as polysaccharides, glycoproteins, or glycolipids. Lectins play a fundamental role in biological recognition on both cellular and molecular level [53]. After initial mucosal cell-binding, lectins can remain on the cellular surface or conduct receptor mediate adhesion, and further go through an internalization process [54]. Such process can provide dual functions including target specific attachment and conduct controlled drug delivery of macromolecular pharmaceuticals via active cell-mediated drug uptake [4]. Lectins will partially suffer from detachment by shed off mucus, however, due to the reversible interaction between lectins and mucins, this detachment can facilitate the distribution of lectins to free lectin receptors on the cell membrane. Once the lectins are bind to the membrane, internalization will immediately occur which makes the free lectin binding site available again [55]. These features can promote the intercellular uptake of drugs from lectins-based formulations. Although lectins have many advantages related to mucoadhesion, some of them have problems such as toxic, immunogenic, and unknow effects from repeated exposure [56].

1.4.2.2. Bacterial invasins. A typical feature of pathogens is the ability of invading and translocating through the epithelial barrier. In bioadhesion systems, this process can also be referred as the term “bioinvasion”. Bacterial invasion into cells is a crucial process of avoiding an attack of the host immune system and initiating transcytosis and multiplication in a suitable environment, which results in the establishment and maintenance of infection [57]. Pathogenic

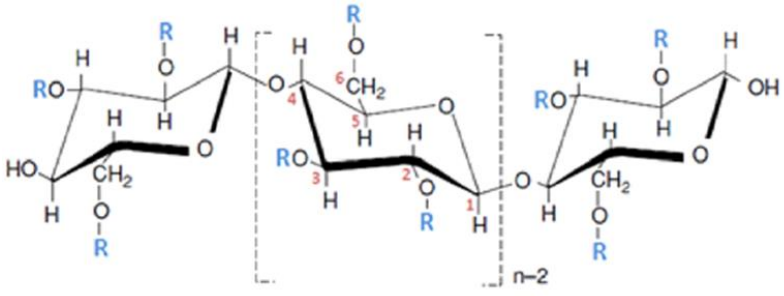
bacteria can adhere to the cell surfaces in gastrointestinal tract with the help of their fimbriae, which are long protein with adhesins found on the surface of many bacterial strains. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are enteropathogens which can reach to the lamina propria from the intestinal lumen by means of entering and passing through intestinal epithelial cells and cause a variety of diseases. Utilizing their unique features, a study has reported on the surface functionalization of liposomes with an invasion protein (InvA497) derived from *Yersinia pseudotuberculosis* as a promising strategy for intracellular drug delivery [58]. The invasive of *Yersiniu* as well as other types of invasins established new approaches for the development of bioinvasive drug delivery systems [59]. Therefore, drug delivery systems based on the special characteristics of bacterial are theoretically efficient to enhance the adhesion and delivery of the drugs.

1.4.2.3. Thiomers. Thiolation opens a new era for mucoadhesive polymers which may enhance the mucoadhesive property for both natural and synthetic polymers. A large variety of polymers have been thiolated such as PAA [60], chitosan [61], CMC [62], xyloglucan [63], and hyaluronic acid [64]. Thiomers or thiolated polymers can be achieved by fix thiol-bearing functional groups on the backbone of well-established polymers [49, 65]. The thiol groups can form strong covalent disulfide bonds with cystine-rich regions of mucous membrane, which can significantly improve the mucoadhesive properties especially in comparison with traditional mucoadhesive materials. Such mechanism of thiomers mimics the nature of secreted mucus glycoproteins, which are also covalently anchored in the mucous membrane by the formation of disulfide bonds [65]. The degree of crosslinking is associated with inter and intra disulfide bond formation, which is controllable by altering the amount of free thiol moieties [66]. Moreover,

thiomers can significantly improve permeability, inhibit efflux pumping, and act as a protection especially for peptides and proteins from enzymatic attack [67, 68].

1.4.3. HPMC

HPMC is a traditional pharmaceutical excipient with an irreplaceable role in the development of pharmaceutical technologies. It plays a key role in mucoadhesive drug delivery systems with massive therapeutic applications. It is not only extensively used as mucoadhesive polymer but also widely exploited in controlled release matrix systems. HPMC belongs to the category of semisynthetic derivative of cellulose, with favorable non-toxic and hydrophilic property. The structure of HPMC consists a basis of a linear polysaccharide cellulose chain with ether-linked methoxy and hydroxypropyl side groups (Figure 1.6) [69]. By altering its chemical structure (substitution degrees and ratios), numerous viscosity grades and molecular weights are available in the market which offer a great variability in its physicochemical properties [70]. Moreover, the swelling and wetting ability of HPMC are important factors affecting the mucoadhesive strength and duration of the interaction.



$$R = -H, -CH_3 \; or \; -(OCH_2CHCH_3)OH$$

Figure 1.6. Chemical structure of HPMC.

Table 1.2
Recent Development of HPMC-Based Formulations (Adapted from [71])

HPMC type, other excipients	Drug	Dosage form	References
HPMC K4M, CP 934P, NaCMC	Felodipine, pioglitazone		[72]
	Diltiazem hydrochloride		[73]
HPMC K4M, CP 934P	Itraconazole		[74]
	Piroxicam		[75]
HPMC K4M/K15M, CP 974P	Sumatriptan succinate	Tablet	[76]
HPMC K4M, CP 974P	Clotrimazole		[77]
HPMC K4M, CP 934P, sodium alginate	Prochlorperazine		[78]
HPMC K15M, CP 940P, NaCMC, sodium alginate	Promethazine hydrochloride		[79]
HPMC K15M, CP 934P, NaCMC	Candesartan		[80]
HPMC K4M, CP 934P (NaHCO3, citric acid)	Venlafaxine hydrochloride	Floating tablet	[81]
HPMC K4M/K100M, PVP K30	Losartan sodium		[82]
HPMC, PVA, NaCMC	Flurbiprofen		[83]
HPMC K4M/K15M, CP 940P, poloxamer 407	Lidocaine	Patch	[84]
HPMC K4M, K15M/ PVP K30/NaCMC	Acyclovir		[85]
HPMC E4M/Eudragit® RLPO, PVP, MC, HPC, chitosan	Naproxen		[86]

(Table 1.2 Continued)

HPMC, Eudragit® RS 100	Didanosine		[87]
HPMC K4M, PVA	Ciprofloxacin hydrochloride		[88]
HPMC K100, PVP	Mefenamic acid		[89]
HPMC K4M, PVA, PEO	Rizatriptan benzoate	Film	[90]
HPMC K15M, K100M, CP 940	Ivabradine hydrochloride		[91]
HPMC E15, CP 934P, PVP K30	Lycopene		[92]
HPMC E50, CMC, MC, CP 934, sodium alginate	Indomethacin	Microcapsule	[93]
HPMC K100, PLA, PEO	Cisplatin		[94]
HPMC K4M, E4M, sodium alginate, chitosan, k-carrageenan	Glutamine	Nanofiber	[95]
HPMC K100M, PLGA	Sitagliptin	Nanoparticle	[96]

Note. CMC = carboxymethylcellulose; PVP = polyvinylpyrrolidone; PVA = polyvinyl alcohol; MC=methylcellulose; HPC = hydroxypropyl cellulose; PEO= poly (ethylene oxide); PLA= polylactic acid; PLGA= poly (lactic-co-glycolic acid).

The mucoadhesion mechanism of HPMC polymer includes the formation of hydrogen bonds from massive hydroxyl groups in its structure and interpenetration of polymer chains with mucins. Belonging to the subset of non-ionic polymers, it is beneficial to avoid the risk of drug interactions and normally has great reproducibility in drug release profiles, as it cannot be significantly influenced by the pH of the environment [71, 97]. Excellent compatibility with other excipients results in many possible applications of HPMC. As a mucoadhesive excipient, mucosae of oral cavity and the whole gastrointestinal tract are the important sites for HPMC-

based systems to exert their excellent mucoadhesive properties [3, 98, 99]. Modern mucoadhesive formulations utilizing HPMC, including tablets, films/patches, nanoparticles, microparticles and nanofibers. Examples of formulations in recent development exploiting the combination of HPMC and other polymers are listed in Table 1.2.

In this study, HPMC was selected as a model polymer due to its mucoadhesive property, extensively application in the pharmaceutical field, non-ionic and inert property, biocompatibility and biodegradability, and the availability of a variety of grades.

1.5. Research Objectives

Different mucoadhesives and the composition of mucous membrane have significant impact on the mucoadhesive performance and its underlying mechanisms. In this study seven different grades of HPMC (K3, K5, K15M, E3, E5, E50, and E4M) are selected as the mucoadhesives to evaluate their influence on different biological membranes. The first objective of this research is to investigate the roles of physicochemical properties of the selected mucoadhesive polymers, including viscosity, surface hydrophilicity and hydration behavior on *in vitro* mucoadhesive performance using buccal, sublingual, stomach, and intestine mucosa of pigs. The purpose is to understand the factors that impact of the mucoadhesive characteristics of HPMC series products.

In order to gain a better understanding of mucoadhesion mechanisms, the theory of Lewis acid-base approach is applied to estimate the surface energy and free energy of adhesion of HPMC compacts in different conditions. So, the second objective of this study to apply the Lewis acid-base theory to explain mucoadhesion.

The third objective of this study is to further inspect the effect of change of surface hydrophilicity on *in vitro* mucoadhesion performance. For the purpose of altering the surface

hydrophilicity, EC is selected as an additive to change surface hydrophilicity and HPMC grade K15M as a testing material.

CHAPTER 2: THE RELATIONSHIP BETWEEN PROPERTIES OF HPMC AND *IN VITRO* MUCOADHESION

2.1. Introduction

Mucoadhesion can be described as the adhesion between polymeric material and a biological mucosal surface. The success of mucoadhesion depends on the retention of the mucoadhesive polymeric material on the mucous membrane [100]. Thus, the property of the chosen material as well as the biochemical properties of the mucous membrane from different body regions play important roles on mucoadhesive performance.

The three major factors that impact on mucoadhesion are environment, physiological condition, and mucoadhesive material. The influence of these factors on mucoadhesion have been described in section 1.3. From the formulation point of view, the nature of the polymer material presents in the formulation is an important factor for mucoadhesion. Therefore, the selected physicochemical properties of polymer such as molecular weight (viscosity), surface hydrophilicity, and hydration behavior have been investigated in this study.

Mucoadhesive performance can be characterized by testing the adhesion strength by *in vitro* and *in vivo* tests. The most employed *in vitro* tests are tensile strength test, shear strength test, peel strength test, *in vitro* retention time, and rheological methods [5]. Texture analyzer is a convenient instrument to characterize the tensile strength between mucoadhesive polymer and certain surface of substrate. In this method, the mucoadhesion was evaluated by measuring the maximum force required to separate the polymer away from the surface of substrate after contact at pre-determined time and force. The work of adhesion can be achieved from specific computation. Currently there is not a single universally accepted parameter for mucoadhesion

test using texture analyzer. The test parameter (e.g. contact time, contact pressure, test speed, and test environment) for texture analysis vary in different published studies [101-103]. Thus, it would be complicated to compare the result of mucoadhesion test from different experimental conditions [104]. But it is still widely used for its simple operation and relative comparability. Several model substrates can be selected while using texture analyzer, such as mucin disc, mucin gel, porcine tissue, chicken pouch tissue, and bovine mucosa etc. The anatomical and physiological similarity of pig to human have been evaluated for decades by scientists, and pig is a large animal with substantial mucous membrane [105-107], thus it is chosen in this study.

In this chapter, the impact of viscosity, surface hydrophilicity, and hydration behavior of different grades of HPMC compacts and the lipid composition of mucous membranes on *in vitro* mucoadhesion were investigated. Texture analyzer was used to measure the force of mucoadhesion, and porcine mucosae were selected as the substrates.

2.2. Materials and Methods

2.2.1. Materials

HPMC (K3, E3, E5, K4M, E4M, and K15M) were purchased from Dow Company. HPMC E50 was purchased from Spectrum Chemical. The properties of different HPMC grades are listed in Table 2.1. Sodium chloride and sodium hydroxide was purchased from VWR. Potassium chloride was purchased from Mallinckrodt Chemicals. Disodium phosphate, monopotassium phosphate, potassium phosphate monobasic and sodium phosphate dibasic was purchased from Spectrum Chemical. Sodium taurocholate was purchased from EMD Millipore. Lecithin was purchased from MP Biomedicals. Maleic acid was purchased from EMD Millipore. Table 2.2-Table 2.4 listed the composition of simulated saliva (SS), fasted state simulated gastric fluid (FaSSGF), and fasted state simulated intestinal fluid (FaSSIF). Porcine

Table 2.1
Properties of Different HPMC Grades [108]

Grade	HPMC	Average viscosity (mPa·s 2% in water at 20°C)	Methoxy substitution (%)	Hydroxypropyl substitution (%)
K	K3 LV	3	19.0-24.0	7.0-12.0
	K4M	4,000		
	K15M	15,000		
E	E3 LV	3	28.0-30.0	
	E5 LV	5		
	E50 LV	50		
	E4M	4,000		

Note. "LV" refers to low viscosity.

Table 2.2
Composition of Simulated Saliva (SS) [109]

Composition	
Sodium chloride (g/L)	8.00
Potassium phosphate monobasic (g/L)	0.19
Sodium phosphate dibasic (g/L)	2.38

Note. Adjust pH to 6.8 with phosphoric acid.

Table 2.3
Composition of Fasted State Simulated Gastric Fluid (FaSSGF) [109]

Composition	
Sodium taurocholate (μM)	80
Lecithin (μM)	20
Pepsin (mg/mL)	0.1
Sodium chloride (mM)	34.2

Note. Adjust pH to 1.6 with hydrochloric acid/sodium hydroxide.

Table 2.4
Composition of Fasted State Simulated Intestinal Fluid (FaSSIF) [109]

Composition	
Sodium taurocholate (mM)	3
Lecithin (mM)	0.2
Maleic acid (mM)	19.12
Sodium hydroxide (mM)	34.8
Sodium chloride (mM)	68.62

Note. Adjust pH to 6.5 with hydrochloric acid.

buccal, sublingual, and intestinal tissues were obtained from a local slaughterhouse (Long Ranch, Manteca, CA). Porcine stomach tissue was purchased from Animal Technologies, Inc.

2.2.2. Preparation of Polymer Compacts

Polymer compacts were prepared using a Carver Press (Carver, Inc., Wabash, IN) with a 10 mm diameter punch and die set. The applied loads were kept constant at 5 metric tons, pressed for 30 sec and ejected from the die. The resulting compacts had an average weight of 500 ± 5 mg. The polymer compacts were stored in a desiccator until further use.

2.2.3. Contact Angle Measurement

The apparent contact angle was measured by sessile drop method with a goniometer (Model G-I, Kernco Instruments Co., Inc., El Paso, TX). The apparent contact angles were measured by applying an aliquot (5 µl) of simulated body fluids (SS, FaSSGF, FaSSIF) on the surface of polymer compacts.

2.2.4. Mucosae Preparation

Porcine buccal and sublingual mucosae were isolated after the removal of fatty layers by surgical scissors, then cleaned with phosphate buffered saline (PBS, pH 7.4) containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4. Porcine mucosae were rinsed by

PBS to remove the particles. All mucosae were rapidly frozen to −80°C for storage and thawed at the time of use at ambient temperature.

2.2.5. Force of Mucoadhesion Measurement

Mucoadhesion testing of the polymer compacts was carried out using a CT3 texture analyzer (Brookfield Engineering Labs, Inc., Middleboro, MA). The polymer compact was attached to the cylindrical probe (10 mm in diameter) by double-sided adhesive tape. The compact was pre-hydrated in normal saline for 0 and 5 minutes before conducting the experiment. The mucosa (about 60 × 60 mm) was equilibrated in the test medium for 15 min before clamped on to the stationary platform. The probe was lowered at a speed of 1.00 mm/s to contact the mucosa with force of 1 g and with contact time 60 s. It was removed at the speed of 4.50 mm/s. Data collection and calculations were performed using TexturePro CT V1.8 Build 13 software. The areas under the load vs. distance curves (AUC in mJ) were determined to represent the maximum force required for detachment of the two systems (mucous membrane/polymer compacts), the data was recorded as force of mucoadhesion. Experiments were run in sextuplicate under room temperature and for each set of measurements a fresh piece of mucosa was used.

2.2.6. Hydration Study

The polymer compacts of 10 mm in diameter were prepared and placed in 20 mL beaker containing 10 mL normal saline as the swelling medium. The temperature was maintained at 37±1℃ using a water bath. The weight of the compacts was measured and recorded after pre-determined time intervals. The hydration (H) was calculated by using the following formula:

$$H = \frac{W_{Hydrated} - W_{Dry}}{W_{Dry}} \times 100\% \quad (2.1)$$

Where W_{Dry} and $W_{Hydrated}$ are the weight of polymer compacts before and after hydration, respectively.

2.3. Results and Discussion

2.3.1. The Relationship between Contact Angle and Viscosity

The apparent contact angles were measured according to section 2.2.3. Contact angle is a common parameter that describes the hydrophilicity/hydrophobicity of a solid surface and provides the information about the wettability of a surface of interest. Wetting is associated with the study of how a liquid spread out on a solid substrate or the formation of boundary surfaces between liquid and solid states. The solid surface with water contact angle less than 90° indicates that the surface is hydrophilic or has better wettability, whereas a solid surface with water contact angle larger than 90° is considered as hydrophobic or non-wettable [110, 111]. Contact angle has different metastable states, which can also be referred as the term hysteresis phenomenon. Such phenomenon has two components: thermodynamic and dynamic. The first one is affected by the roughness and heterogeneity of the surface [112], while the second one depend on time, chemical interaction of liquid-material, penetration of the measuring liquid into the pores, particle reorganization on the surface [113-115]. In this study, the measurement of apparent contact angle was conducted instantaneously after placing the liquid on the compact surface to minimize the dynamic component of hysteresis.

The contact angle values of HPMC compacts were in the range of 50° to 80°, which indicated the surface HPMC compacts are hydrophilic in nature. In Figure 2.1, a strong correlation has been found between contact angle and viscosity, an increase in the viscosity of HPMC (molecular weight) resulted in higher contact angle values. This correlation implied that contact angle is related to the degree of polymerization of HPMC. For two types of polymers

almost having the same viscosity value but different grades, the "E" grade exhibited slightly higher contact angle values than the "K" grade. Although they both have same range of

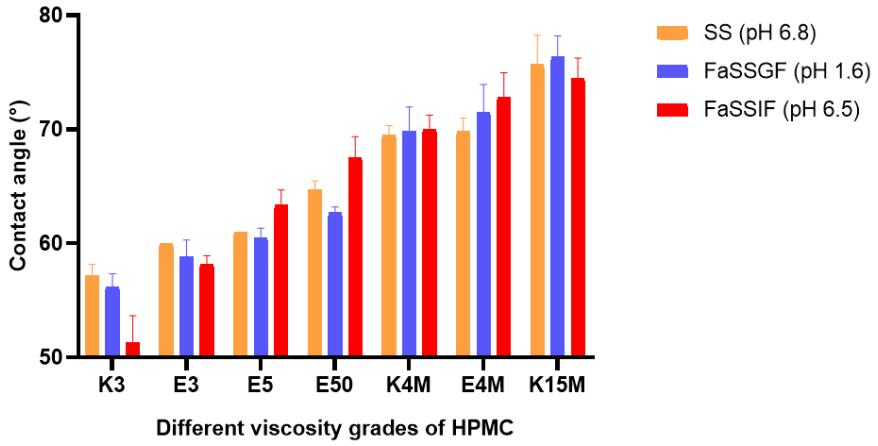


Figure 2.1. The correlation between different viscosity grades of HPMC and contact angle measured with various body fluid (SS, FaSSGF, FaSSIF) on dry compacts. (n=6)

substitute ratio of hydroxypropyl ($-OCH_2CHOHCH_3$) groups (in the range of 7.0% to 12.0%), "E" grade has 28.0% to 30.0% of methoxy ($-OCH_3$) groups while "K" grade has 19.0% to 24.0% of methoxy groups. The concentration of methoxy group is directly related to the viscosity/inflexibility of HPMC. Higher concentration of methoxy group results in a more viscous or less flexible state. Therefore, more percentages of methoxy groups in "E" grade might resulted in an increase in the viscosity, thus further lead to marginally higher contact angle values.

In addition, as studied by Joshi et al., the increasing viscosity of HPMC can lead to a reduction in the porosity of the tablet/compact, which indicates a decrease in the surface roughness [116]. As mentioned previously, surface roughness is a considerable factor affecting the outcome of contact angle. Wenzel [117] stated that adding surface roughness will enhance

the wettability caused by the chemistry of the surface. For example, for a given contact angle less than 90°, increase surface roughness of the same solid material will result in a smaller contact angle; whereas, if a liquid forms a contact angle of greater than 90° on a smooth solid, increase surface roughness of the same solid material will result in a larger contact angle [118]. In this study, the contact angle values of compacts were all below 90°, thus the HPMC compacts with smaller viscosity might possess a rougher surface with increased porosity, and further give rise to smaller contact angle values.

Different simulated body fluids have different pH values: the pH value of FaSSGF is 1.6, the pH value of FaSSIF is 6.5, and the pH value of SS is 6.8. While measuring with these three different types of simulated body fluids, no significant difference in contact angle values was shown in each individual grade. This phenomenon reflected the insensitivity of HPMC to the pH alteration, which is beneficial for its application in a variety of physiological environment. Such characteristic attributed to the non-ionic feature of HPMC.

2.3.2. The Relationship between Contact Angle and Force of Mucoadhesion

Force of mucoadhesion was measured as described in section 2.2.5. Four different types of porcine mucosae were selected as the model substrates: buccal, sublingual, stomach, and intestinal mucosa. The measured force of mucoadhesion between the compacts and (1) buccal mucosae were in the range of 0.63 mJ to 2.53 mJ; (2) sublingual mucosae were in the range of 0.50 mJ to 2.61 mJ; (3) stomach mucosae were ranged from 0.89 mJ to 1.88 mJ; (4) intestinal mucosae were in the range of 0.07 mJ to 0.51 mJ, respectively.

As shown in Figure 2.2, the force of mucoadhesion increased with the increasing viscosity of HPMC, regardless of the type of mucosa used for measurement. This suggested that the viscosity of the polymer has a significantly impact on the mucoadhesive performance. Of the

mucosal surfaces evaluated, oral region exhibited better mucoadhesive performance, while intestinal mucosae showed the lowest mucoadhesive force among all different grades of HPMC.

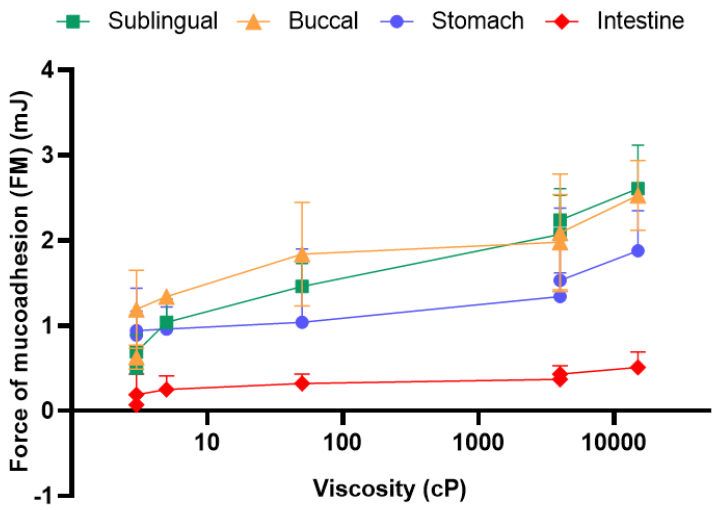


Figure 2.2. The correlation between viscosity and force of mucoadhesion (FM) on porcine mucosae. Results measured on dry HPMC compacts. (n=6)

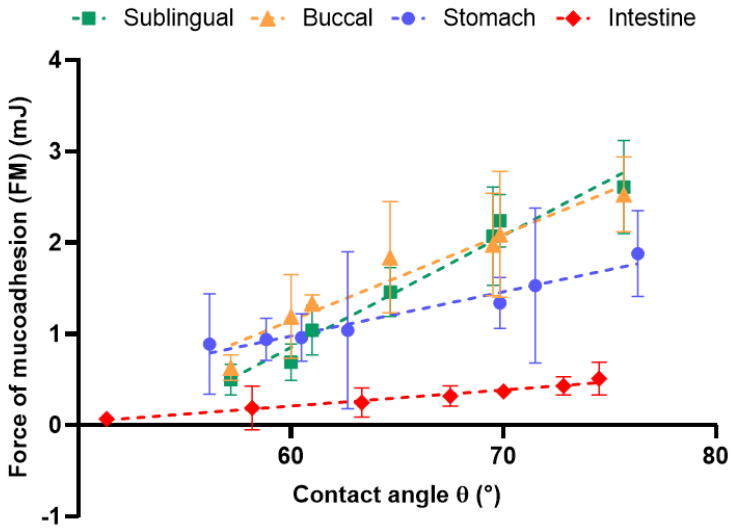


Figure 2.3. The correlation between contact angle (θ) and force of mucoadhesion (FM) on porcine mucosae. Results measured on dry HPMC compacts. (n=6)
Sublingual: $y = 0.1224x - 6.493$ ($R^2 = 0.9762$); buccal: $y = 0.09449x - 4.523$ ($R^2 = 0.9386$); stomach: $y = 0.04847x - 1.937$ ($R^2 = 0.9501$); intestine: $y = 0.01764x - 0.8478$ ($R^2 = 0.9743$). Linear regression was analyzed by GraphPad Prism 8.

This might be related with the differences in the biochemical property of each type of mucosa from different body regions.

Recall that in section 2.3.1, a positive correlation was found between viscosity and contact angle values. Thus, as shown in Figure 2.3, the correlation between contact angle and force of mucoadhesion is exhibited. The force of mucoadhesion increased linearly as the contact angle values increased, which denoted that there is a strong positive correlation between these two factors. Generally speaking, the increasing of contact angle values would indicate decreasing wettability of the solid surface, which further leads to a decrease in the adhesive strength [119, 120]. However, this positive correlation showed a contrary fact. The reason for the increasing contact angle values, as discussed before, might be due to the HPMC compacts surface became less rough and tended to be hydrophobic with the increasing viscosity. This contrary fact suggested that the mucoadhesive force was prone to be regulated by other factors rather than the surface hydrophilicity, which further implied that the effects of initial contact stage was masked from the effects of the latter consolidation stage of mucoadhesion. Therefore, it appeared to be inappropriate to interpret the mucoadhesive performance only from the surface hydrophilicity/hydrophobicity property.

2.3.3. The Impact of Lipid Content on Mucoadhesive Performance

As shown in Figure 2.2 and Figure 2.3, the mucoadhesive force exhibited differently on different types of issues. This phenomenon was most likely attributed to the varieties in biochemistry property of different types of mucosae. The mucosa is an assembly of similar cells and their extracellular matrix. The basic structure of cell membrane consists of lipid molecules forming a self-assembly lipid bilayer, with hydrophobic tails shielding from the water in the interior, and the hydrophilic headgroups exposing to the exterior [121]. In this case, when

placing a polymer compact on a piece of mucosa, the polymer compact can actually interact with the hydrophilic headgroups of cell membrane on a micro level. The hydrophilic headgroups and hydrophobic tails are composed of polar lipids and non-polar lipids, respectively.

Hence, the lipid content of each mucosa from literatures was organized as shown in Table 2.5 and Figure 2.4. It is obvious that the amount of polar lipids and the proportional of polar lipids to non-polar lipids in the mucosae were increasing in the rank of sublingual, buccal, stomach and intestine. In addition, the polar lipids were the main contribution to the increasing total lipid amount, thus the total lipids amount in each mucosa were also increasing with the aforementioned ranking order. Each slope from Figure 2.3 ($\Delta FM/\Delta\theta$) can be defined as the rate of mucoadhesive force increases with the increasing viscosity of HPMC for each type of mucosal surface. Such defined parameter ($\Delta FM/\Delta\theta$) showed negative linear correlation with the percentage of polar lipid content (Figure 2.5). From this "coincidence", it can be deduced that the mucoadhesive force might be altered by the proportion of polar lipids in the mucosa with a pattern: with the increasing viscosity of HPMC, the less the proportion of polar lipids in the mucosa, the larger the increasing rate of mucoadhesive force.

Owing to the polar lipids are the basis of forming the hydrophilic regions of cell membrane and the hydrophilic regions are facing outwards to the external environment, it is reasonable that the polar lipids can interact with the polymer and further influence the mucoadhesive force. Due to the natural of polar lipids, they are more favorable to interact with polymer with charges. However, HPMC belongs to the category of non-ionic polymers, thus the increasing of polar lipids did not promote the mucoadhesive interactions, instead, it hampered the interactions and weaken the mucoadhesive property of HPMC. Furthermore, when develop

mucoadhesive dosage forms for regions with high proportion of polar lipids, such as the intestinal region, ionic polymers might be a preferable choice as the mucoadhesive material.

Table 2.5
Lipid Content (mg/g dry) of Different Types of Mucosae [122-125]

	Different types of mucosae			
	Sublingual	Buccal	Stomach	Intestine
Total lipid	79.4	130.0	134.0	350.0
Non-polar lipid	30.6	35.3	29.6	63.1
Polar lipid	48.8	95.2	104.4	286.9
Polar lipid/non-polar lipid	1.6	2.7	3.5	4.5

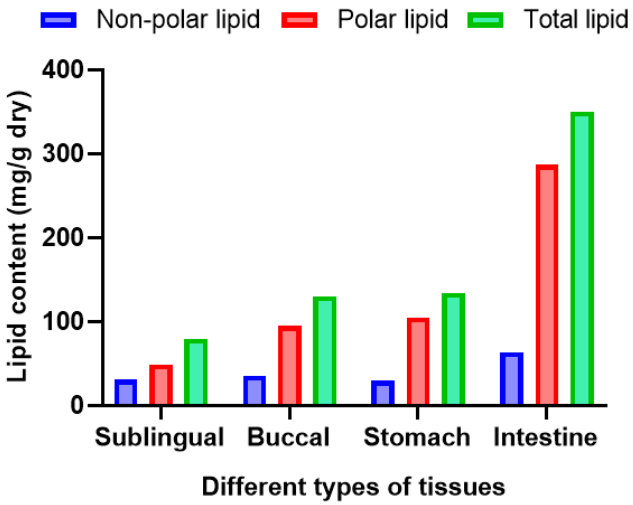


Figure 2.4. The lipid content of different types of mucosa (buccal, sublingual, stomach and intestine).

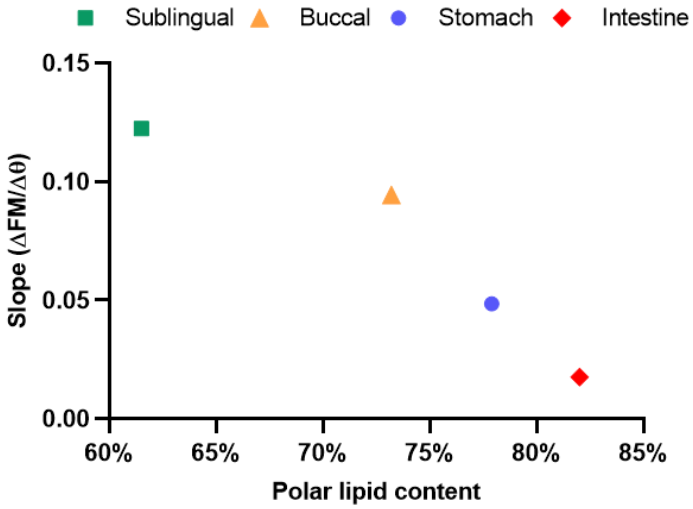


Figure 2.5. The correlation between slope of $\Delta FM/\Delta\theta$ and polar lipid content. Pearson $r = -0.9470$. Correlation was analyzed by GraphPad Prism 8.

2.3.4. Hydration Behavior

Hydration behavior was measured by means of section 2.2.6. It was found that 5 min is enough to show the impact of hydration on in vitro mucoadhesive performance in our preliminary investigation. Therefore, 5 min was selected as the pre-hydration time.

The hydration behavior of HPMC is closely related to its structure and molecular weight. From chemistry point of view, the interaction between HPMC and water is mainly affected by the formation of hydrogen bonding. Based on the structure of HPMC, hydroxyl group acts as an electron acceptor when interacting with a water molecule, while methoxy groups act as an electron donor within hydrogen bonds [126].

The hydration behavior of different grades of HPMC compacts was shown in Figure 2.6. After pre-hydrating the HPMC compacts for 5 min, the hydration percentage showed an increase with the increasing of viscosity grades, which indicated the improved water uptake ability. The highest hydration percentage was seen at the highest viscosity grade, which was K15M. This

hydration profile implied that the ability of dehydrating a mucus layer was promoted with the increasing viscosity in a short period of time.

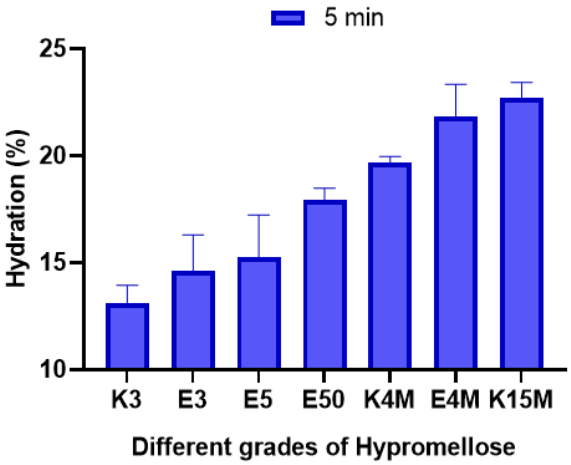


Figure 2.6. Hydration behavior of HPMC compacts in normal saline at 5 minutes. (n=3)

A layer of mucus gel sits on the mucosal surface which mainly contains over 95% of water and 0.2%-5% of mucin. Mucus gel is sandwiched between mucosal epithelial cell surface and the mucoadhesive in a mucoadhesive joint, and it was recognized that water displacement from the mucosal surface is a requirement for the material to form mucoadhesion. A substantial amount of water movement between a dry or partially hydrated mucoadhesive and a contacting mucosal surface could increase the cohesive and adhesive properties of the mucus gel, which further strengthen the mucoadhesive joint [127]. In this study, the aforementioned higher mucoadhesive force of HPMC may be due to its better ability of dehydrating the mucus gel to form a stronger adhesive joint, thus this provides a possible explanation to the result of improved mucoadhesive force with increasing viscosity.

The correlation between mucoadhesive force and hydration time was shown in Figure 2.7-Figure 2.10. After the pre-hydration process, most of the mucoadhesive forces decreased to

less than 0.5 mJ. Highest mucoadhesion was observed when compacts were in dry state, however, the mucoadhesive force reduced with the pre-hydration process. Mucoadhesive materials need to absorb water for binding, while surrounding by massive amount of water, the hydroxyl and methoxy groups in HPMC are attracted to water molecules, thus not sufficient amount of hydroxyl and methoxy groups are remained to conduct hydrogen bonding with mucus layer. Therefore, it was found that restricted hydration was required for stable long term mucoadhesion [128]. As the hydration of the compacts increased, the dehydration of mucus gel became more and more difficult. With the pre-hydration process, the polymer chain segments could be over extended and had less flexibility for interpenetration and entanglement resulting in lower mucoadhesion [129].

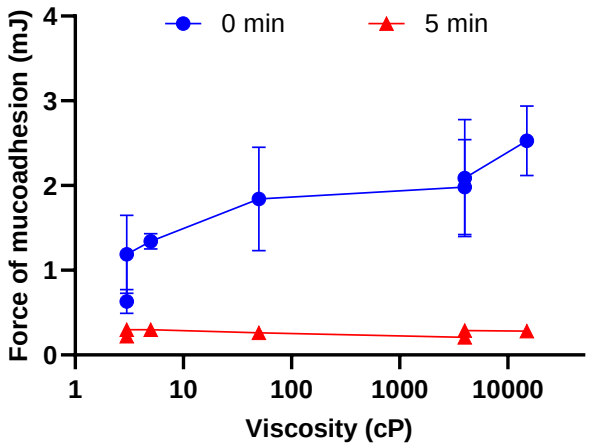


Figure 2.7. Force of mucoadhesion of different viscosity grades of HPMC compacts measured with porcine buccal mucosae in SS at different hydration levels. Legends showing different time points in minutes. (n=6)

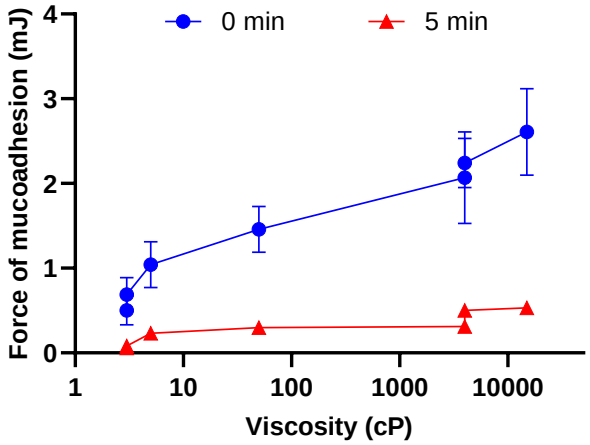


Figure 2.8. Force of mucoadhesion of different viscosity grades of HPMC compacts measured with porcine sublingual mucosae in SS at different hydration levels. Legends showing different time points in minutes. (n=6)

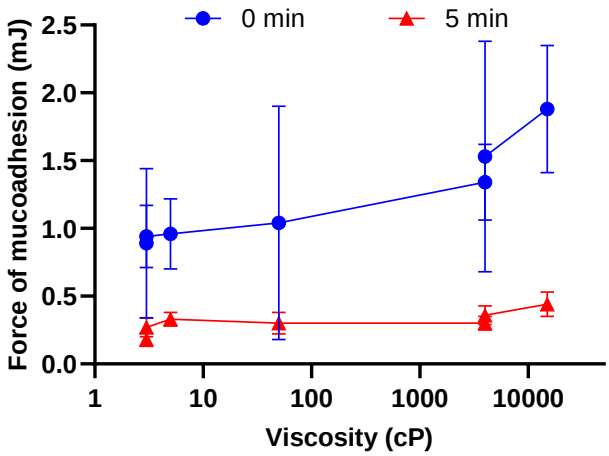


Figure 2.9. Force of mucoadhesion of different viscosity grades of HPMC compacts measured with porcine stomach mucosae in FaSSGF at different hydration levels. Legends showing different time points in minutes. (n=6)

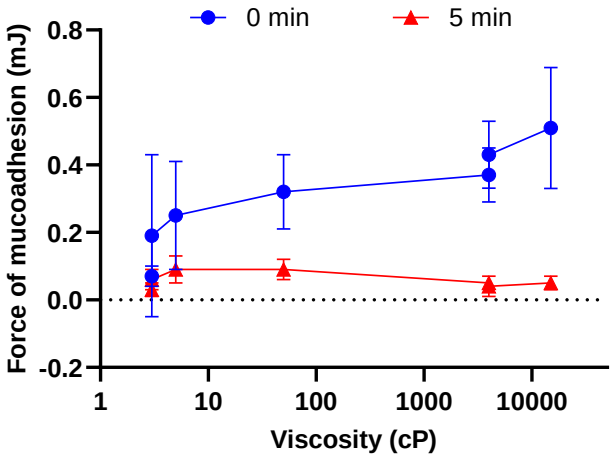


Figure 2.10. Force of mucoadhesion of different viscosity grades of HPMC compacts measured with porcine intestinal mucosae in FaSSIF at different hydration levels. Legends showing different time points in minutes. (n=6)

2.4. Summary

In this chapter, the surface hydrophilic property of HPMC was determined by their contact angle values. Positive correlation between contact angle values and viscosity values of HPMC was found suggesting that the degree of polymerization of HPMC affected the surface hydrophilicity. Moreover, smaller viscosity grades of HPMC compacts lead to relatively greater surface roughness and further resulted in smaller contact angle values. Additional investigation is needed to establish this observation.

The pH of the simulated body fluids did not significantly fluctuate the contact angle values, which attributed to the natural non-ionic property of HPMC. Such property promoted the broadly use of HPMC under diverse physiological situations.

The mucoadhesive force of HPMC on different types of mucosae suggested positive correlations with the viscosity values of HPMC. Moreover, the mucoadhesive force data measured on each mucosa were found positively correlated to the corresponding contact angle

values. Therefore, the mucoadhesive force was likely more impacted at consolidation stage than the initial contact stage.

The amount of polar lipids in each mucosa contributed to different mucoadhesive performance for HPMC compacts. The less the polar lipid amount, the better the mucoadhesive performance with greater viscosity grade of HPMC compacts. For regions with abundant amount of polar lipids, mucoadhesive polymer with charges would be a preferable choice than non-ionic polymers.

The pre-hydration behavior has increased with the increasing viscosity of HPMC. The mucoadhesive performance of HPMC compacts has significantly weakened after the pre-hydration process, which indicated that restricted hydration was considered as a crucial prerequisite for a prolonged and stable mucoadhesion process.

CHAPTER 3: THERMODYNAMIC ANALYSIS OF MUCOADHESION ON POLYMER COMPACTS

3.1. Introduction

During the development of mucoadhesive drug delivery system, the selection of mucoadhesive material in the formulation is always one of the major concerns for pharmaceutical scientists. In order to find materials with better mucoadhesive properties, it is essential to have a better understanding of mucoadhesion mechanisms. Mucoadhesion is a complex phenomenon, and as mentioned in the section 1.2, six theories have been proposed to describe the integrate process of mucoadhesion, including wetting theory, diffusion interlocking theory, electronic theory, adsorption theory, mechanical theory, and facture theory. One single theory may not able to describe the mucoadhesion phenomenon completely. However, a combination of these possible theories can lead to an explanation at different stages of the interactions between mucoadhesive materials and mucous membrane. The basic mechanism of mucoadhesion is commonly accepted as two stages: wetting or swelling of the mucoadhesive material, and interpenetration and formation of bonds between mucoadhesive and mucin chains [5]. A lot of researches have focused on these two stages to interpret the mucoadhesion phenomenon.

The interpenetration and interdiffusion mechanism have been supported by experimental studies with different methods. The spectroscopic analysis has been applied to study the interpenetration between mucoadhesive materials and mucin chains successfully, especially the ATR-FTIR spectroscopy [130-133]. The confocal laser scanning microscopy can also examine the penetration of fluorescent labelled polymers into the mucus gel layer [134]. Other indirect

studies for interpenetration is based on the rheological behavior at the interface of mucus gel and mucoadhesive. It was found that the molecular interpenetration can lead to rheological synergism between the mucoadhesive and mucosal surface, which would further consolidate the adhesive binding [135, 136].

The surface chemistry and surface free energy of materials contribute to the performance of many processes and products, one of which is mucoadhesion in the pharmaceutical field [137]. Thermodynamic analysis of surface energy can be used to study the mechanism of wetting, which is known as the driving force of mucoadhesion. The Lewis acid-base theory from Van Oss, Chaudhury and Good is the most developed model to interpret the principles related to the thermodynamic surface properties of solid. The progenitor of this theory was the division of surface free energy into Lifshiz-Van der Waals interaction (γ^{LW}) and acid-base interaction (γ^{AB}). The Lifshiz-Van der Waals interaction originates from the molecular theory of intermolecular forces between nonpolar molecules. The acid-base interaction is mostly occurred by hydrogen bonding, especially in many nonmetallic condensed materials [138]. Due to the uniqueness of the acid-base interaction, the surface parameter is expressed using two terms: electron acceptor (γ^{+}) and electron donor (γ^{-}). For a surface of interest, these surface parameters (γ^{LW}, γ^{+} and γ^{-}) can be quantified by contact angle measured with three specific liquid (one apolar liquid and two polar liquids), and the interfacial free energy in a binary system as well as the free energy of adhesion in a ternary system can be further calculated. A lot of examples showed that there is good correlation between total free energy of adhesion (ΔG^{TOT}) and the measured force of mucoadhesion [139-143]. However, wetting process only contributes to the initial step of mucoadhesion, thus thermodynamic analysis of mucoadhesion may be insufficient to describe the full process of mucoadhesion in some cases.

In this study, thermodynamic analysis of HPMC compacts (seven viscosity grades) was conducted by the Lewis acid-base approach in order to reveal the mucoadhesion mechanism of HPMC compacts. This investigation further examined if the experimental data are consistent with the computation results via the Lewis acid-base approach.

The impacts of viscosity grades and contact angle values of HPMC on their mucoadhesive performance is established in this study (chapter 2). Among different grades of HPMC, grade K15M showed the maximum mucoadhesive force. A combination polymer compacts of EC with viscosity of 10 cP and HPMC K15M were chosen in this study. EC, very similar to HPMC, is also one of the cellulose derivatives. Its structure contains repeating glucose units with some of the hydroxyl groups converted into ethyl ether groups (Figure 3.1). Due to the ethyl groups in its composition, it is not water soluble. EC belongs to the category of non-ionic polymer, the hydroxyl groups on its structure are behaved as mucoadhesive functional groups. The ratio of EC in the combination compact was up to 40% due to the highest percentage of EC in solid dosage forms is normally no more than 40% [137-139]. The effects of physicochemical properties of mixed polymer compacts on the mucoadhesion was also studied from a thermodynamic point of view.

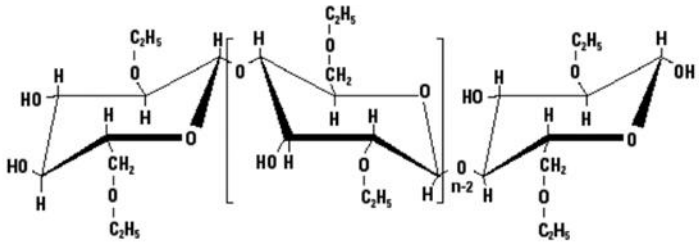


Figure 3.1. Chemical structure of EC.

3.2. Theory of Lewis Acid-Base Approach

The characterization of surface free energy components of solids and interfacial interactions are recognized as the key to understand the mechanism of surface-based phenomena. Lewis acid-base approach was developed from Van Oss, Chaudhury and Good to understand the theoretical principles related to thermodynamic surface properties of solids [138]. Based on Fowkes principle, the total surface energy (γ^{TOT}) is divided into two components: Lifshiz-van der Waals interaction and Lewis acid-base interactions [139]. It can be expressed as follows:

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \tag{3.1}$$

Where, $\gamma^{LW}$is Lifshiz-van der Waals interactions (apolar component); γ^{AB} results from Lewis acid-base interactions (polar component).

According to Dupre, when two unlike bodies i and j are brought together reversibly, the total free energy of adhesion ΔG_{ij} for the interface between condensed phases i and j is given by [140]:

$$\Delta G_{ij} = \gamma_{ij} - \gamma_i - \gamma_j \tag{3.2}$$

$$\Delta G_{ij} = \Delta G_{ij}^{LW} + \Delta G_{ij}^{AB} \tag{3.3}$$

The Lifshiz-van der Waals interaction, which can also be referred as the apolar component, it is the lump of intermolecular interactions which includes London dispersion force, Debye force and Keesom force. London dispersion force have been considered as the predominate interactions between macroscopic bodies in condensed systems. Berthelot proposed a geometric mean combining rule for intermolecular interactions due to the symmetry of the London dispersion force [140]. According to its rule, the apolar component of surface free energy (γ_{ij}^{LW}) and free energy of adhesion (ΔG_{ij}^{LW}) can be achieved:

$$\gamma_{ij}^{LW} - \gamma_i^{LW} - \gamma_j^{LW} = -2\sqrt{\gamma_i^{LW}\gamma_j^{LW}} \tag{3.4}$$

$$\gamma_{ij}^{LW} = \left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_j^{LW}}\right)^2 \tag{3.5}$$

$$\Delta G_{ij}^{LW} = -2\sqrt{\gamma_i^{LW}\gamma_j^{LW}} \tag{3.6}$$

For acid-base component, the molecular interacting of the free energy of adhesion and of interfacial tension between two phases is mostly occurred by hydrogen bonding. The Lewis acid-base theory is more commonly used to describe hydrogen bonding. In an acid-base interaction, a complementary of functions exists due to the lack of symmetry. Thus, the acid-base parameter γ^{AB} is separate into two distinct parameters: electron acceptor γ^+ (Lewis acid) and electron donor γ^- (Lewis base) component [140].

For a pure substance, the value of γ^{AB} can be calculated by the following equation:

$$\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-} \tag{3.7}$$

The acid-base component of surface free energy γ_{ij}^{AB} and free energy of adhesion ΔG_{ij}^{AB} is given by:

$$\gamma_{ij}^{AB} = 2(\sqrt{\gamma_i^+\gamma_i^-} + \sqrt{\gamma_j^+\gamma_j^-} - \sqrt{\gamma_i^+\gamma_j^-} - \sqrt{\gamma_i^-\gamma_j^+}) \tag{3.8}$$

$$\Delta G_{ij}^{AB} = \gamma_{ij}^{AB} - \gamma_i^{AB} - \gamma_j^{AB} \tag{3.9}$$

$$= -2(\sqrt{\gamma_i^+\gamma_j^-} + \sqrt{\gamma_i^-\gamma_j^+})$$

On combining equation (3.6) and (3.9), the total free energy of adhesion ΔG_{ij} between substances i and j is given by:

$$\Delta G_{ij} = \Delta G_{ij}^{LW} + \Delta G_{ij}^{AB} \tag{3.10}$$

$$= -2(\sqrt{\gamma_i^{LW}\gamma_j^{LW}} + \sqrt{\gamma_i^+\gamma_j^-} + \sqrt{\gamma_i^-\gamma_j^+})$$

According to Young-Dupre equation, when a liquid (l) is placed on the surface of an ideal solid (s), the free energy of adhesion across the interface (ΔG_{sl}) can be determined by measuring contact angles (θ) of the liquid on the surface of the solid:

$$\Delta G_{sl} = -\gamma_l(1 + \cos\theta) \tag{3.11}$$

$$= -2(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^+\gamma_l^-} + \sqrt{\gamma_s^-\gamma_l^+})$$

If three liquids (l_1, l_2, l_3) form non-zero contact angles ($\theta_i, i = 1, 2, 3$) on the surface of a solid, a set of general contact angle equations can be obtained:

$$\gamma_{l_1}(1 + \cos\theta_1) = 2(\sqrt{\gamma_s^{LW}\gamma_{l_1}^{LW}} + \sqrt{\gamma_s^+\gamma_{l_1}^-} + \sqrt{\gamma_s^-\gamma_{l_1}^+}) \tag{3.12a}$$

$$\gamma_{l_2}(1 + \cos\theta_2) = 2(\sqrt{\gamma_s^{LW}\gamma_{l_2}^{LW}} + \sqrt{\gamma_s^+\gamma_{l_2}^-} + \sqrt{\gamma_s^-\gamma_{l_2}^+}) \tag{3.12b}$$

$$\gamma_{l_3}(1 + \cos\theta_3) = 2(\sqrt{\gamma_s^{LW}\gamma_{l_3}^{LW}} + \sqrt{\gamma_s^+\gamma_{l_3}^-} + \sqrt{\gamma_s^-\gamma_{l_3}^+}) \tag{3.12c}$$

For a non-polar liquid l_1, γ_s^{LW} can be obtained by using equation (3.12a):

$$\gamma_s^{LW} = \gamma_{l_1}^{LW}\frac{(1+\cos\theta_1)^2}{4} \tag{3.13}$$

Combining equations (3.12b), (3.12c) and (3.13), the values of γ_s^+ and γ_s^- can be solved:

$$\sqrt{\gamma_s^+} = \frac{AF-BD}{CF-DE} \tag{3.14}$$

$$\sqrt{\gamma_s^-} = \frac{BC-AE}{CF-DE} \tag{3.15}$$

Where,

$$A = \gamma_{l_2}(1 + \cos\theta_2) - 2\sqrt{\gamma_s^{LW}\gamma_{l_2}^{LW}} \tag{3.16}$$

$$B = \gamma_{l_3}(1 + \cos\theta_3) - 2\sqrt{\gamma_s^{LW}\gamma_{l_3}^{LW}} \tag{3.17}$$

$$C = 2\sqrt{\gamma_{l_2}^-} \tag{3.18}$$

$$D = 2\sqrt{\gamma_{l_2}^+} \tag{3.19}$$

$$E = 2\sqrt{\gamma_{l_3}^-} \tag{3.20}$$

$$F = 2\sqrt{\gamma_{l_3}^+} \tag{3.21}$$

When the surface of material 1 (e.g. adhesive) is placed on the surface of material 2 (e.g. mucous membrane) immersed in a liquid 3 (e.g. biological fluid), the free energy of adhesion involved apolar (ΔG^{LW}) and polar (ΔG^{AB}) component can be calculated by the following equations [141, 142]:

$$\Delta G^{AB} = \gamma_{12}^{AB} - \gamma_{13}^{AB} - \gamma_{23}^{AB} \tag{3.22}$$

$$= 2\left[\sqrt{\gamma_3^+}\left(\sqrt{\gamma_1^-} + \sqrt{\gamma_2^-} - \sqrt{\gamma_3^-}\right) + \sqrt{\gamma_3^-}\left(\sqrt{\gamma_1^+} + \sqrt{\gamma_2^+} - \sqrt{\gamma_3^+}\right) - \sqrt{\gamma_1^+\gamma_2^-} - \sqrt{\gamma_1^-\gamma_2^+}\right]$$

$$\Delta G^{LW} = \gamma_{12}^{LW} - \gamma_{13}^{LW} - \gamma_{23}^{LW} \tag{3.23}$$

$$= \left(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}}\right)^2 - \left(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_3^{LW}}\right)^2 - \left(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_3^{LW}}\right)^2$$

Thus, the total free energy of adhesion can be obtained:

$$\Delta G^{TOT} = \Delta G^{LW} + \Delta G^{AB} \tag{3.24}$$

$$= \gamma_{12} - \gamma_{13} - \gamma_{23}$$

$$= \gamma_{12}^{LW} - \gamma_{13}^{LW} - \gamma_{23}^{LW} + 2\left[\sqrt{\gamma_3^+}\left(\sqrt{\gamma_1^-} + \sqrt{\gamma_2^-} - \sqrt{\gamma_3^-}\right) + \sqrt{\gamma_3^-}\left(\sqrt{\gamma_1^+} + \sqrt{\gamma_2^+} - \sqrt{\gamma_3^+}\right) - \sqrt{\gamma_1^+\gamma_2^-} - \sqrt{\gamma_1^-\gamma_2^+}\right]$$

3.3. Calculation of Theoretical Hydroxyl Values of HPMC

HPMC can be considered as homopolymer, the number of hydroxyl groups can be obtained with a theoretical estimation assuming that the three substitutions (either a methyl, hydroxypropyl groups or a hydrogen atom) on HPMC are equally distributed in the polymer structure (recall Figure 1.6). In this case, two hydroxyl groups are theoretically on one repeating unit. HPMC has a linear structure as one of the cellulose derivatives [144], thus per polymer chain contains two ending groups. From the structure of HPMC, it should be noticed that one ending group has three hydroxyl groups. In general, the estimated hydroxyl group number (N) per polymer chain can be calculated as:

$$N = (n - 2) \times 2 + 2 \times 3 \tag{3.25}$$

Where, n is the sum of repeating units and ending groups ($n \geq 3$).

The molecular weight of one repeating unit or one ending group can be calculated based on the structure of HPMC. By knowing the molecular weight of different grades of HPMC, the mole number of repeating units in one mole of the polymer can further be quantified as follows:

$$Mole\ of\ repeating\ units = \frac{M_n \times 1 - M_{eg} \times 2}{M_R} \tag{3.26}$$

Where, M_n is the molecular weight of the polymer; M_{eg} is the molecular weight of each ending group; M_R is the molecular weight of each repeating unit.

By using equation (3.25) and the mole number of repeating units, the number of hydroxyl groups in one gram of polymer can be calculated as follows:

$$N = \frac{1}{Mn} \times (mole\ of\ repeating\ units \times 2 + 2 \times 3) \times 6.02 \times 10^{23} \tag{3.27}$$

3.4. Materials and Methods

3.4.1. Materials

Glycerol (99.5+%) and diiodomethane (99%) were purchased from Sigma-Aldrich Corporation. Ethyl cellulose 10 cP was purchased from DOW company. Others were the same as in section 2.2.1.

3.4.2. Preparation of HPMC and Combination Compacts

Different grades of HPMC compacts and combination compacts of HPMC K15M and EC were prepared. The method used to prepare polymer compacts has been described in section 2.2.2. The relative composition of the two polymers in the combination compacts ranged from 100/0-60/40 in K15M/EC weight percent ratio.

3.4.3. Contact Angle Measurement

The method used to measure contact angle has been described in section 2.2.3. The apparent contact angles were measured by applying an aliquot (5 μl) of water, glycerol (GL) or diiodomethane (DIM) on the surface of polymer compacts, respectively. Surface energy parameters of Lifshiz-van der Waals and the Lewis acid-base interactions of the polymer compacts were calculated using equation (3.12a)-equation (3.12c) based on contact angles of two polar liquids (water and GL) and one apolar liquid (DIM). The interfacial free energy of mucoadhesion in a binary system, which consists of polymer and water, was calculated by using equation (3.10). The free energy of mucoadhesion in a ternary system, which consists of mucin, polymer, and corresponding media (gastric fluid, normal saline, and intestinal fluid), was calculated by using equation (3.24). The surface energy parameters of water, glycerol, diiodomethane, mucin, gastric fluid, normal saline, and intestinal fluid applied in the calculation are listed in Table 3.1.

3.4.4. Force of Mucoadhesion Measurement

The method used to measure force of mucoadhesion of the polymer compacts has been described in section 2.2.5.

Table 3.1
Surface Energy Parameters (in mJ/m^2) of Various Materials [145]

	γ	γ^{LW}	γ^{AB}	γ^{+}	γ^{-}
Water	72.80	21.80	51.00	25.50	25.50
Glycerol (GL)	64.00	34.00	30.00	3.92	57.40
Diiodomethane (DIM)	50.80	50.80	0.00	-	-
Mucin	46.20	6.92	39.28	49.17	7.84
Gastric fluid (pH 1.2)	75.90	40.40	35.50	5.80	54.70
Saline (pH 6.4)	74.50	28.80	45.70	6.90	75.80
Intestinal fluid (pH 7.5)	75.70	47.10	28.60	1.70	122.40

3.5. Results and Discussion

3.5.1. Thermodynamic Analysis of HPMC Compacts

In order to probe the mucoadhesion mechanism of HPMC compacts from a thermodynamic point of view, the surface energy analysis with Lewis-acid base approach was utilized. The apparent contact angles of water (θ_{water}), glycerol (θ_{GL}) and diiodomethane (θ_{DIM}) on the surface of HPMC compacts were calculated as shown in Table 3.2. All the contact angle values were measured on dry polymer compact surfaces. The results showed that the standard deviation of the mean angles was between 1-3° for most of the compacts. The contact angles of θ_{water} and θ_{GL} increased whereas θ_{DIM} decreased with the increase of viscosity of HPMC.

Table 3.2
The Apparent Contact Angles (°) of Water (θ_{water}), Glycerol (θ_{GL}), and Diiodomethane (θ_{DIM}) on the Surface of Different Grades of HPMC Compacts

HPMC	θ_{water}	θ_{GL}	θ_{DIM}
K3	50.67±0.52	53.50±1.22	24.50±0.84
E3	53.33±0.82	57.33±1.37	20.00±1.10
E5	56.50±0.55	60.00±1.10	19.50±2.17
E50	58.17±1.98	61.17±0.98	18.17±1.47
K4M	61.00±1.55	62.67±1.21	17.83±1.33
E4M	62.83±0.75	63.67±0.82	16.33±1.21
K15M	68.67±1.21	67.00±0.89	14.33±0.82

Note. N=6.

Table 3.3
Single Component of Surface Energy Parameters (in mJ/m^2) for HPMC Compacts

HPMC	$\sqrt{\gamma_s^+}$	$\sqrt{\gamma_s^-}$	γ_s^+	γ_s^-	γ_s^{LW}	γ_s^{AB}	γ^{TOT}
K3	0.09	5.40	0.01	29.12	46.33	0.94	47.27
E3	-0.21	5.33	0.04	28.42	47.78	-2.24	45.55
E5	-0.33	5.12	0.11	26.16	47.93	-3.37	44.56
E50	-0.39	4.97	0.15	24.72	48.30	-3.85	44.45
K4M	-0.42	4.69	0.17	21.96	48.39	-3.89	44.50
E4M	-0.45	4.49	0.20	20.20	48.77	-4.06	44.71
K15M	-0.54	3.88	0.29	15.09	49.23	-4.20	45.03

Using the contact angle data in Table 3.2, the surface energy parameters for each grade of HPMC compacts were calculated based on Lewis acid-base approach as shown in Table 3.3. It was noticed that most values of $\sqrt{\gamma_s^+}$ and γ_s^{AB} for HPMC compacts (except K3) were negative.

Negative square roots, especially in the calculation of γ_s^+, were mostly exclusive for contact angles results. This can be explained by the experimental error in the measurement of contact angles. Good and Van Oss [140] noticed that a not implausible "correction" in one or more observed contact angles could eliminate small negative magnitude of $\sqrt{\gamma_s^+}$. Another consideration is that for solids with relatively low hydrophilicity, the contact angle of water might show a time dependent behavior, and eventually cause the presence of this error. Consider the negative values of $\sqrt{\gamma_s^+}$ are empirically valid, equation (3.7) from section 3.2 must be written in the form below under this circumstance:

$$\gamma^{AB} = 2\sqrt{\gamma^+}\sqrt{\gamma^-} \tag{3.7b}$$

The significance of $\sqrt{\gamma_s^+}$ can be interpreted as the acid character of the solid surface results in a negative contribution to the total surface energy (γ_s^{TOT}) of the solid [139]. Using equation (3.7b), the result of γ^{AB} might be a negative value. For a mechanically stable condensed system, the negativity of γ^{AB} is physically acceptable if $\gamma^{AB} < \gamma^{LW}$, given that the total surface energy can remain positive [140, 143].

As shown in Table 3.3, the values of γ_s^- were relatively larger in comparison with the values of γ_s^+ in all polymer compacts. Several possible explanations could explain this result. Firstly, due to the lone pair of electrons on the oxygen atom of the hydroxyl and methoxy groups, the HPMC showed predominantly more electron-donor tendency in dry state [146]. Secondly, it was pointed out that in certain carbohydrate structures, all the hydroxyl groups are pointed "inward" away from the adjacent phase. This is caused by the formation of hydrogen bonding by Lewis-neutralization between these hydroxyl groups and the Lewis base oxygen atoms of the adjacent hydroxyl groups. When stronger Lewis base is presented in the other phase, it might be expected that the those hydroxyl groups bonded with oxygen atoms can be

attracted by the external Lewis base, thus the hydroxyl groups can turn from "inward" to "outward" orientation. Another possible explanation is for hydrated surface, the water molecules may bond to the surface enough tightly that they cannot easily desorb, and the Lewis acid character may not be apparent [140]. In this study, it is not suitable to apply the last explanation as all the compacts were in dry state.

The profiles of calculated interfacial free energy in a binary system and free energy of adhesion in a ternary system are depicted in the following content. The calculation of interfacial free energy between HPMC compacts and four different media, including gastric fluid (ΔG_{PG}), normal saline (ΔG_{PS}), intestinal fluid (ΔG_{PI}), and water (ΔG_{PW}), were all negative (Table 3.4). With the increasing viscosity of HPMC, the values of ΔG_{PG}, ΔG_{PS}, ΔG_{PI} and ΔG_{PW} calculated from four corresponding media were increasing as shown in Table 3.4. Negative value of ΔG indicates the spontaneous formation of adhesive joint in a certain system, the more negative value of ΔG will imply higher potential of forming adhesive binding [140, 143]. Therefore, the potential of forming adhesive bond between polymer compacts and different media declined as the viscosity of HPMC increased. The correlation of contact angles of water and ΔG_{PW} between polymer compacts and water is shown in Figure 3.2. The contact angle of water and ΔG_{PW} showed a positive correlation with the increasing viscosity of HPMC. Increasing ΔG_{PW} implies the potential of forming adhesive joints decrease between HPMC compacts and water, which also indicated the decreased surface wettability. This result was consistent with the increasing contact angles of water. Figure 3.3-Figure 3.6 showed the correlations of ΔG_{PG}, ΔG_{PS} and ΔG_{PI} with mucoadhesive force measured between HPMC compacts and different types of mucosae (buccal, sublingual, stomach and intestine). In all four figures, as the viscosity increased, an increase in ΔG_{PG}/ΔG_{PS}/ΔG_{PI} and the corresponding increase in force of mucoadhesion measured

on four types of mucosae was observed. This result agreed with the positive correlations found between mucoadhesive force and contact angles in section 2.3.2.

Table 3.4
The Calculated Interfacial Free Energy Between Different Grade of HPMC Compacts and Different Media (ΔG, in mJ/m^2)

HPMC	ΔG_{PG} (pH 1.2)	ΔG_{PS} (pH 6.4)	ΔG_{PI} (pH 7.5)	ΔG_{PW}
K3	-113.69±0.95	-102.89±1.21	-109.18±2.03	-118.94±0.51
E3	-110.32±1.20	-98.51±1.44	-103.89±2.14	-116.27±0.83
E5	-107.65±0.95	-95.40±1.24	-100.83±1.86	-112.98±0.58
E50	-106.44±0.93	-93.93±0.95	-99.53±1.56	-111.20±1.06
K4M	-104.73±0.99	-92.01±1.26	-98.26±2.51	-108.09±1.72
E4M	-103.62±0.73	-90.66±0.85	-97.32±1.46	-106.04±0.85
K15M	-99.78±0.87	-86.25±1.04	-94.21±1.62	-99.28±1.43

Note. N=6. ΔG_{PG}: the interfacial free energy between polymer compacts and gastric fluid; ΔG_{PS}: the interfacial free energy between polymer compacts and normal saline; ΔG_{PI}: the interfacial free energy between polymer compacts and intestinal fluid; ΔG_{PW}: the interfacial free energy between polymer compacts and water.

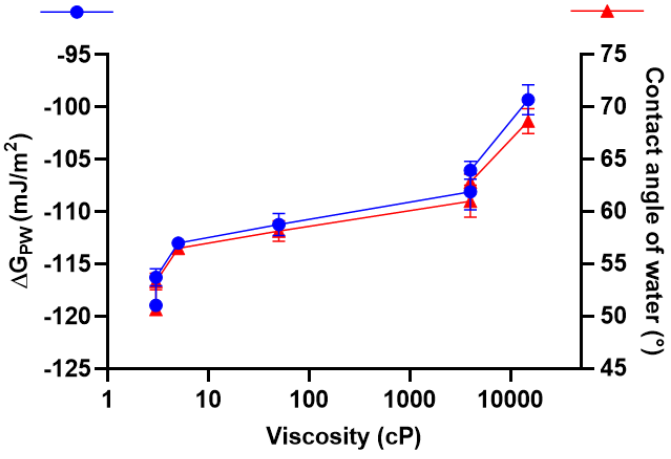


Figure 3.2. The correlation between ΔG_{PW} of HPMC compacts and their contact angle data of water with the increase of viscosity. (n=6)

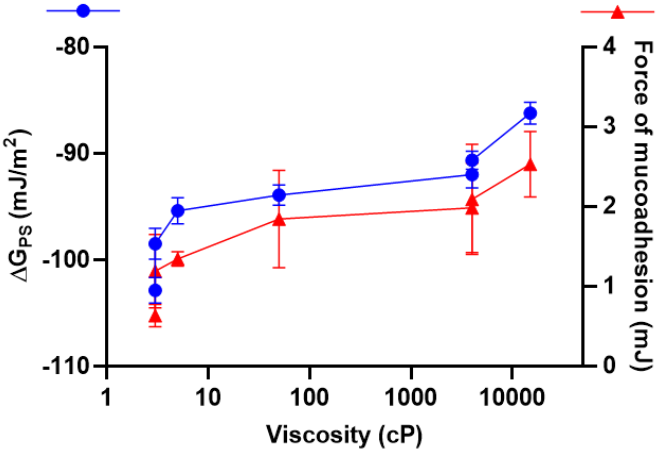


Figure 3.3. The correlation between ΔG_{PS} of HPMC compacts and their force of mucoadhesion data measured on porcine buccal mucosae with the increase of viscosity. (n=6)

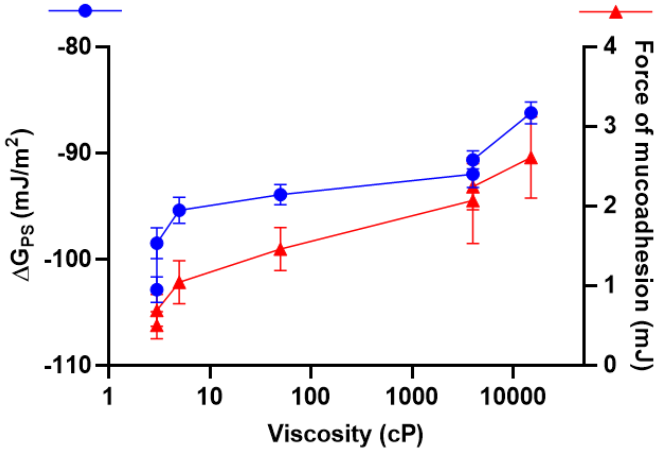


Figure 3.4. The correlation between ΔG_{PS} of HPMC compacts and their force of mucoadhesion data measured on porcine sublingual mucosae with the increase of viscosity. (n=6)

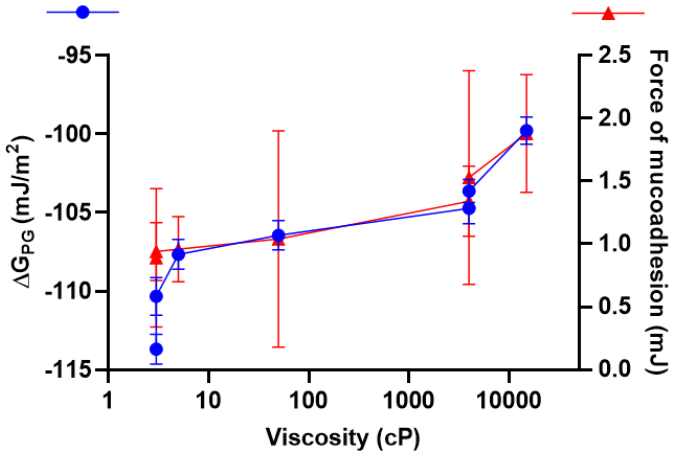


Figure 3.5. The correlation between ΔG_{PG} of HPMC compacts and force of mucoadhesion measured on porcine stomach mucosae with the increase of viscosity. (n=6)

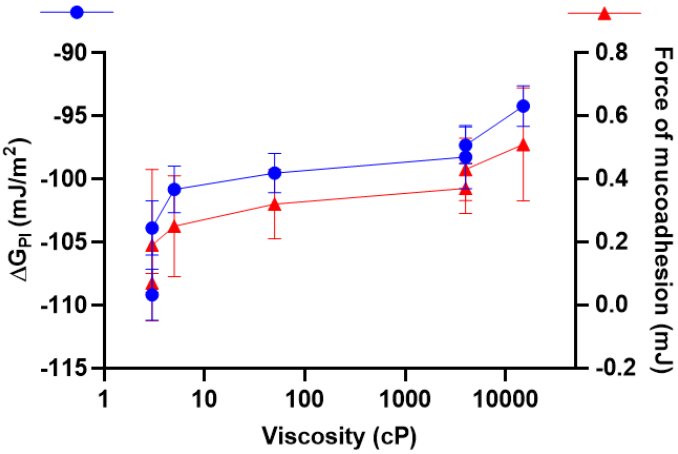


Figure 3.6. The correlation between ΔG_{PI} of HPMC compacts and their force of mucoadhesion data measured on porcine intestinal mucosae with the increase of viscosity. (n=6)

Table 3.5

The Calculated Free Energy of Adhesion among Different Grade of HPMC Compacts, Mucin, and Different Media (ΔG, in mJ/m^2)

HPMC	Gastric fluid (pH 1.2)			Normal saline (pH 6.4)			Intestinal fluid (pH 7.5)		
	ΔG^{LW}	ΔG^{AB}	ΔG^{TOT}	ΔG^{LW}	ΔG^{AB}	ΔG^{TOT}	ΔG^{LW}	ΔG^{AB}	ΔG^{TOT}
K3	3.36±0.16	-2.92±2.27	0.43±2.15	7.88±0.12	-0.97±2.51	6.91±2.42	-0.48±0.18	44.59±3.40	44.11±3.27
E3	4.15±0.18	-5.05±2.00	-0.91±1.97	8.46±0.13	-3.91±2.22	4.55±2.19	0.42±0.20	40.43±3.01	40.85±2.97
E5	4.22±0.33	-4.16±1.82	0.06±1.60	8.52±0.24	-3.42±2.07	5.10±1.90	0.51±0.37	40.92±2.82	41.43±2.56
E50	4.42±0.21	-3.38±1.88	1.05±1.75	8.66±0.15	-2.85±2.04	5.81±1.94	0.73±0.24	41.59±2.74	42.33±2.60
K4M	4.47±0.19	-1.01±3.95	3.46±3.95	8.70±0.14	-0.68±4.20	8.02±4.19	0.79±0.21	44.39±5.62	45.18±5.61
E4M	4.68±0.16	0.43±1.91	5.10±2.02	8.85±0.12	0.57±2.02	9.42±2.10	1.02±0.18	45.98±2.70	47.00±2.83
K15M	4.92±0.09	5.22±2.46	10.14±2.58	9.03±0.07	4.87±2.59	13.90±2.58	1.30±0.10	51.47±3.46	52.77±3.45

Note. N=6

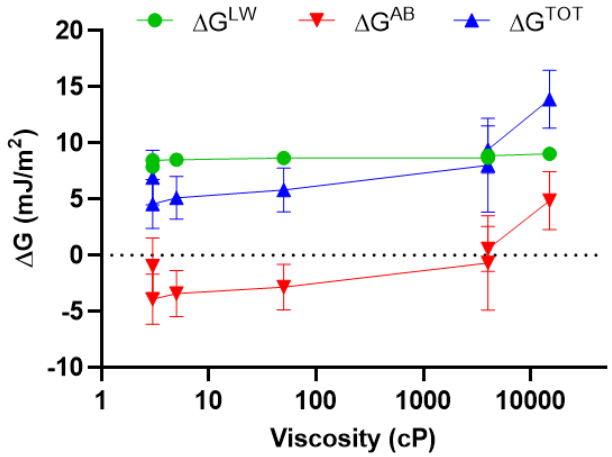
The calculated results of free energy of adhesion among polymer compacts, mucin, and different body fluid (including gastric fluid, normal saline, and intestinal fluid) were shown in Table 3.5. The values of ΔG^{AB} were mostly negative in the environment of gastric fluid and normal saline (except E4M and K15M), while the values of ΔG^{LW} were positive in all the media. ΔG^{TOT} showed the combination effects of these two interactions, which mostly remained positive. This result implied that Lewis acid-base showed potential of driving the spontaneous formation of adhesive bonding in some cases, however, the overall effect indicated that it was less likely to occur in spontaneous mucoadhesion. Figure 3.7A-Figure 3.7C represented the individual influence of ΔG^{LW} and ΔG^{AB} on ΔG^{TOT} in three different media. As the viscosity of HPMC increased, the data of ΔG^{LW} did not change significantly. This suggested that viscosity is not an influencing factor for the van der Waals forces generated between the mucosal surface and the polymer material. However, the van der Waals force exhibited different extent under different pH conditions. ΔG^{LW} was the higher in the normal saline and gastric fluid, while near to zero in the intestinal fluid, which indicated weaker van der Waals force would occur between polymer compacts and mucin with the presence of intestinal fluid.

Furthermore, both the values of ΔG^{AB} and ΔG^{TOT} showed increasing trend respectively among “K” and “E” grades with the increasing of viscosity. The increasing of ΔG^{AB} indicated the contribution from Lewis acid-base interaction was gradually decreasing. For HPMC, both hydroxyl and methoxy groups are the function groups that could induce acid-base interaction. However, the percentage of methoxy groups within different grades of HPMC were in the same range, thus the ΔG^{AB} values might be regulated by the fluctuation in the amount of hydroxyl groups. From the trend of ΔG^{AB}, it is reasonable to deduce that low viscosity grade of HPMC has more hydroxyl groups than the high viscosity grade. As shown in Table 3.6, theoretical

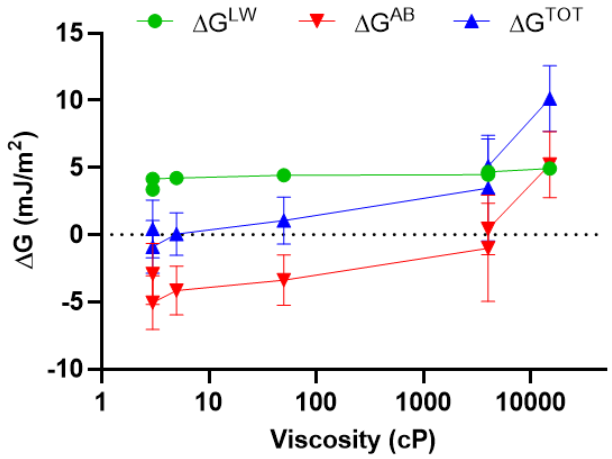
number of hydroxyl groups for different grades of HPMC were calculated according to section 3.3. For both "K" and "E" grades, the theoretical number of hydroxyl groups was declining with the increasing of viscosity, which verified the inference and gave out an explanation for the increasing ΔG^{AB}.

Greater influence of ΔG^{AB} on ΔG^{TOT} was noticed on the HPMC compact surface properties (Figure 3.7A-Figure 3.7C). This implied ΔG^{AB} was the most considerable factor regulating the mucoadhesive force. Since, higher viscosity grades of HPMC exhibited larger mucoadhesive force (section 2.3.2), a positive correlation between ΔG^{AB} and force of mucoadhesion resulted. It was observed that the better mucoadhesive performance from higher viscosity grade of HPMC was not hindered by relatively decreased hydroxyl groups as well as the reduced potential of forming hydrogen bonds (increased ΔG^{AB}). One potential explanation for this is the apparent contact angle was measured instantaneously after placing on the liquid drop. Due to its hysteresis phenomenon, the timing of measurement is one of the important factors which can affect the chemical interactions at the surface-liquid interface and further fluctuate contact angle values. While the complete formation of hydrogen bonds might be a longer period process, the ΔG^{AB} calculated from those apparent contact angles were unlikely to represent the whole process of hydrogen bonding interactions. Therefore, although with reducing potential of forming hydrogen bonds, an increasing mucoadhesive force with increasing viscosity of HPMC was still possible.

A.



B.



C.

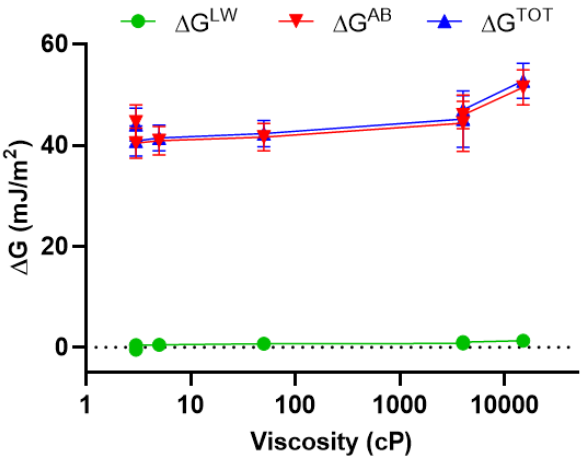


Figure 3.7. Effect of ΔG^{LW} and ΔG^{AB} on ΔG^{TOT} on the HPMC compact surface properties (A. normal saline; B. gastric fluid; C. intestinal fluid). (n=6)

Table 3.6
Calculated Number of Hydroxyl Groups and ΔG^{AB} of Different Media of Different Grades of HPMC

HPMC	Mwt (g/mol) [147]	Number of hydroxyl groups/gram	ΔG^{AB}	ΔG^{AB}	ΔG^{AB}
			Gastric fluid	Normal saline	Intestinal fluid
K grade					
K3	6500	4.92×10^{21}	-2.92±2.27	-0.97±2.51	44.59±3.40
K4M	66900	4.76×10^{21}	0.43±1.91	0.57±2.02	45.98±2.70
K15M	75300	4.76×10^{21}	5.22±2.46	4.87±2.59	51.47±3.45
E grade					
E3	8100	4.89×10^{21}	-5.05±2.00	-3.91±2.22	40.43±3.01
E5	11100	4.85×10^{21}	-4.16±1.82	-3.42±2.07	40.92±2.82
E50	33800	4.78×10^{21}	-3.38±1.88	-2.85±2.04	41.59±2.74
E4M	61800	4.76×10^{21}	-1.01±3.95	-0.68±4.20	44.39±5.62

Note. Grades are in italics.

Positive correlations were observed between ΔG^{TOT} calculated in different media and force of mucoadhesion measured on different types of mucosae (Figures 3.8-Figure 3.11). Higher value of ΔG^{TOT} commonly suggests lower potential of forming adhesive joint, in this case, with the increasing of ΔG^{TOT}, the mucoadhesive force should be decreasing. However, instead of weakening the mucoadhesive property, the mucoadhesive force of HPMC was in fact increasing. It was stated in section 2.2.3 that other factors, such as viscosity and the ability of dehydrating the mucus layer, might be the main contributions to the mucoadhesive performance relative to the surface properties of the compacts. However, the basis of thermodynamic analysis was related to the surface properties of polymer compacts, thus a reasonable deduction is that calculated ΔG^{TOT} could not interpret the mucoadhesive property of HPMC completely. Moreover, the timing effect on contact angle was lack of consideration in this study.

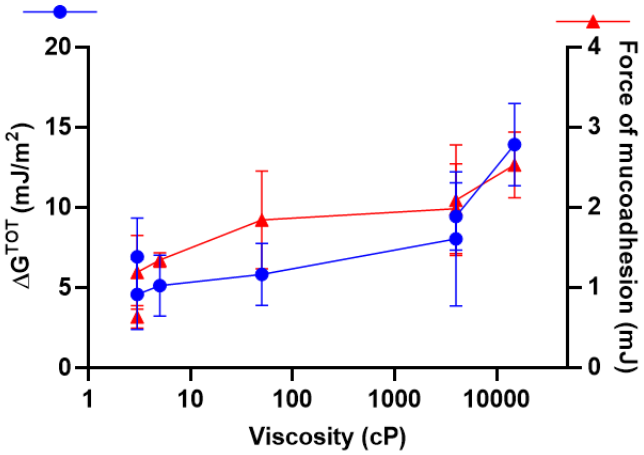


Figure 3.8. The correlation between ΔG^{TOT} of HPMC compacts and their force of mucoadhesion data measured on porcine buccal mucosae with the increase of viscosity. (n=6)

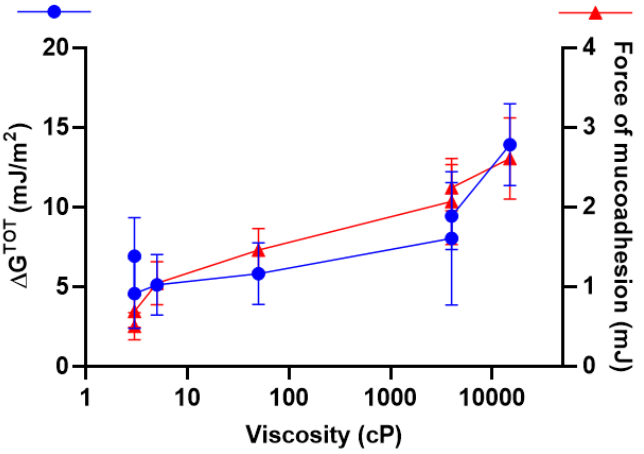


Figure 3.9. The correlation between ΔG^{TOT} of HPMC compacts and their force of mucoadhesion data measured on porcine sublingual mucosae with the increase of viscosity. (n=6)

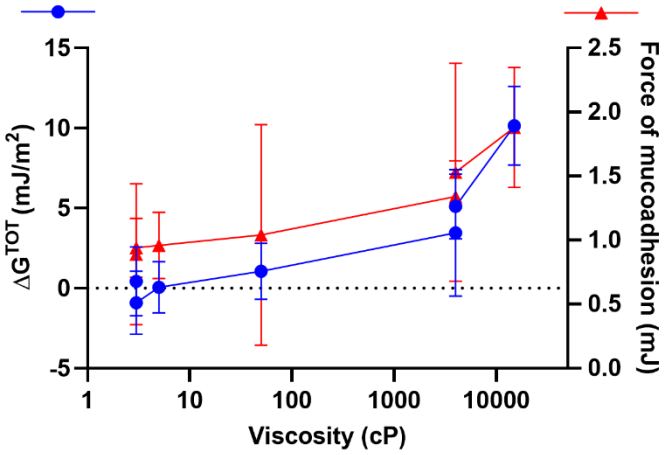


Figure 3.10. The correlation between ΔG^{TOT} of HPMC compacts and their force of mucoadhesion data measured on porcine stomach mucosae with the increase of viscosity. (n=6)

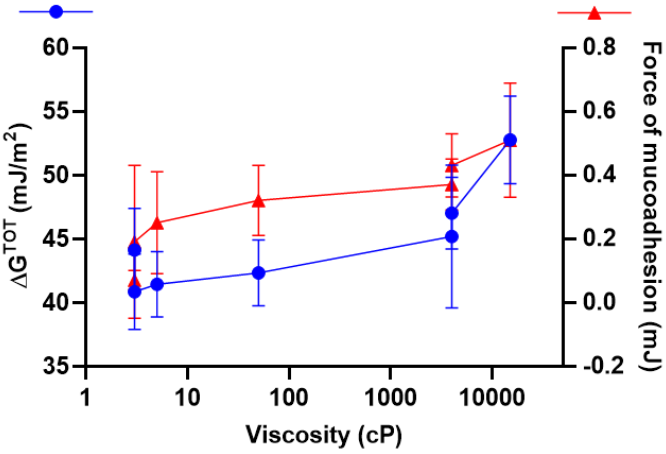


Figure 3.11. The correlation between ΔG^{TOT} of HPMC compacts and their force of mucoadhesion data measured on porcine intestinal mucosae with the increase of viscosity. (n=6)

3.5.2. K15M & EC Combination Compacts

According to the pattern in section 2.3.2, the mucoadhesive force of HPMC compacts increased with the increasing of contact angle values. EC was added into HPMC compacts which had the best mucoadhesive performance (K15M) in order to alter their surface hydrophilicity and contact angles. The contact angles of combination compacts were measured by placing different types of body fluid (SS, FaSSGF and FaSSIF) on the compact surface (same as section 2.2.3). EC is a relatively hydrophobic polymer in comparison with HPMC, thus involving of EC lead to a gradual increase in the contact angle values with the increasing percentage of EC (Table 3.7).

The mucoadhesive force of combination compacts were studied on four different types of porcine mucosal surfaces buccal, sublingual, stomach, and intestine (Figure 3.12). Unlike the results of HPMC compacts, the mucoadhesive force of combination compacts on buccal and sublingual mucosae have decreased with an increase in the amount of EC. For stomach mucosa, the mucoadhesive force showed an increase when involving 10% of EC in the combination compacts, however, the force decreased as EC percentage was increased to 40%. This might because of the low pH value in gastric fluid brought large number of protons and increased the hydrogen bonding efficacy when 10% of EC was involved. However, with the continuous increasing of EC, such effect may be influenced by other factors such as the decreased average viscosity or weakened hydration capacity. Although the mucoadhesive force measured on intestinal mucosa did not display considerable differences among combination compacts, the addition of EC resulted in an overall decrease in mucoadhesive force in relative to compacts with 0% of EC. In general, the mucoadhesive force of combination compacts have decreased with the addition of EC.

Combining the results of contact angle and mucoadhesive force of combination compacts, the possible reasons for the decrease in mucoadhesive property may be their increased hydrophobicity, decreased hydration, and lowered interdiffusion between polymer chains and mucin chains. Therefore, owing to the combination compacts cannot sufficiently dehydrate the mucus layer, and the decreased ability of polymer chains entanglement and interpenetration, their force of mucoadhesion gradually decreased with the increasing percentage of EC in the compacts.

Table 3.7
The Apparent Contact Angles (°) of Different Body Fluids (SS, FaSSGF, FaSSIF) on the Surface of Combination Compacts

EC	SS	FaSSGF	FaSSIF
0%	75.67±2.58	76.33±1.86	74.50±1.76
10%	76.17±1.17	76.50±1.05	75.17±0.75
20%	77.00±1.26	76.83±0.75	75.33±1.21
30%	77.33±1.21	77.67±1.97	76.00±2.10
40%	77.50±1.05	78.33±1.63	76.33±1.37

Note. N=6.

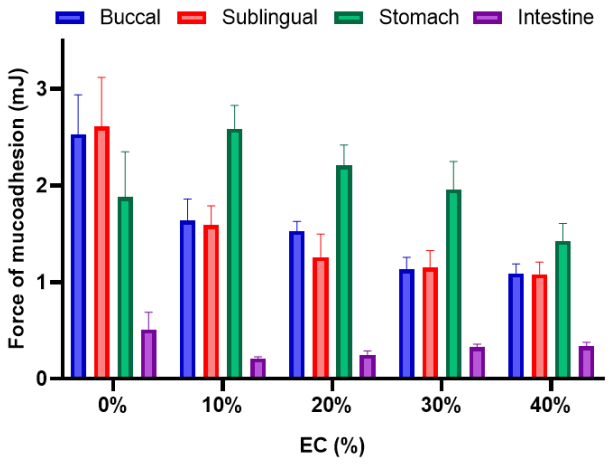


Figure 3.12. The mucoadhesive force of combination compacts measured on porcine buccal, sublingual, stomach, and intestinal mucosae. (n=6)

3.5.3. Thermodynamic Analysis of Combination Compacts

For the purpose of understanding the mucoadhesion mechanism of combination compacts (HPMC grade K15M and EC), thermodynamic analysis via Lewis acid-base approach was conducted. The combination compacts were prepared as described in section 3.4.2. The apparent contact angle values of water, glycerol, and diiodomethane on the surface of dry combination compacts are shown in Table 3.8. The standard deviation of the mean angles was below 2° for all the compacts. Due to the relatively hydrophobic property of EC, the contact angle values showed slightly increasing with increasing percentage of EC.

Using the data from Table 3.8, the surface energy parameters for combination compacts based on Lewis acid-base approach were calculated and the data is shown in Table 3.9. The reasons for the negativity of $\sqrt{\gamma_s^+}$ and γ_s^{AB} as well as the smaller values of γ_s^+ were similar to HPMC compacts as explained in the previously. A decrease of γ^{TOT} with the increasing percentage of EC was seen (Table 3.9). γ^{TOT} could be considered as the indication of the wettability of the solid surface [148, 149]. In this case, for the same kind of media, the wettability of the solid surface decreases with the increasing percentage of EC.

Table 3.8
The Apparent Contact Angles (°) of Water (θ_{water}), Glycerol (θ_{GL}), and Diiodomethane (θ_{DIM}) on the Surface of Dry Combination Compacts of HPMC K15M and EC

EC (%)	θ_{water}	θ_{GL}	θ_{DIM}
0	68.67±1.21	67.00±0.89	14.33±0.82
10	70.00±1.67	69.50±0.84	19.00±1.55
20	70.00±0.63	69.67±1.03	21.67±1.21
30	70.83±1.33	70.67±0.82	23.33±1.37
40	71.17±0.41	72.00±0.89	26.83±0.41

Note. N=6.

Table 3.9
Single Component of Surface Energy Parameters (in mJ/m^2) for Combination Compacts

EC (%)	$\sqrt{\gamma_s^+}$	$\sqrt{\gamma_s^-}$	γ_s^+	γ_s^-	γ_s^{LW}	γ_s^{AB}	γ^{TOT}
0	-0.54	3.88	0.29	15.09	49.23	-4.20	45.03
10	-0.66	3.92	0.43	15.37	48.07	-5.16	42.91
20	-0.63	3.95	0.40	15.59	47.27	-4.99	42.28
30	-0.66	3.92	0.44	15.34	46.73	-5.19	41.54
40	-0.71	4.01	0.50	16.07	45.48	-5.68	39.79

The calculated values of ΔG_{PG}, ΔG_{PS}, ΔG_{PI} and ΔG_{PW} between combination compacts and different media are shown in Table 3.10. As the percentage of EC increases, the hydrophobicity of the surface increases and the wettability decreases, the trend of ΔG_{PG}, ΔG_{PS}, ΔG_{PI} and ΔG_{PW} calculated in different media exhibited increase. A positive correlation has been found between the contact angle of water and ΔG_{PW} with the increasing percentage of EC (Figure 3.13), which revealed the decreasing surface hydrophilicity of combination compacts. The correlation of ΔG_{PG}, ΔG_{PS}, ΔG_{PI} with mucoadhesive force measured on four different types of mucosae (buccal, sublingual, stomach and intestine) was shown in Figure 3.14-Figure 3.17. As the percentage of EC increases, negative correlations between ΔG_{PG}/ΔG_{PS}/ΔG_{PI} and force of mucoadhesion were respectively observed in the figures. The increasing interfacial free energy (ΔG_{PG}/ΔG_{PS}/ΔG_{PI}) represented the decreased potential of forming adhesion joints between combination compacts and corresponding media. The decreasing mucoadhesive force showed an agreement with the interfacial free energy, which implied the mucoadhesive performance of combination compacts can be regulated by the surface energy parameters.

Table 3.10
The Calculated Interfacial Free Energy between Combination Compacts and Different Media (ΔG, in mJ/m^2)

EC (%)	ΔG_{PG} (pH 1.2)	ΔG_{PS} (pH 6.4)	ΔG_{PI} (pH 7.5)	ΔG_{PW}
0	-99.78±0.87	-86.25±1.04	-94.21±1.62	-99.28±1.43
10	-97.17±0.87	-83.52±1.06	-90.58±1.55	-97.70±2.00
20	-96.95±1.00	-83.49±1.20	-90.43±1.80	-97.70±0.76
30	-95.84±0.76	-82.37±0.98	-89.13±1.68	-96.70±1.60
40	-94.43±0.84	-81.06±1.05	-87.08±1.69	-96.30±0.49

Note. N=6. ΔG_{PG}: the interfacial free energy between polymer compacts and gastric fluid; ΔG_{PS}: the interfacial free energy between polymer compacts and normal saline; ΔG_{PI}: the interfacial free energy between polymer compacts and intestinal fluid; ΔG_{PW}: the interfacial free energy between polymer compacts and water.

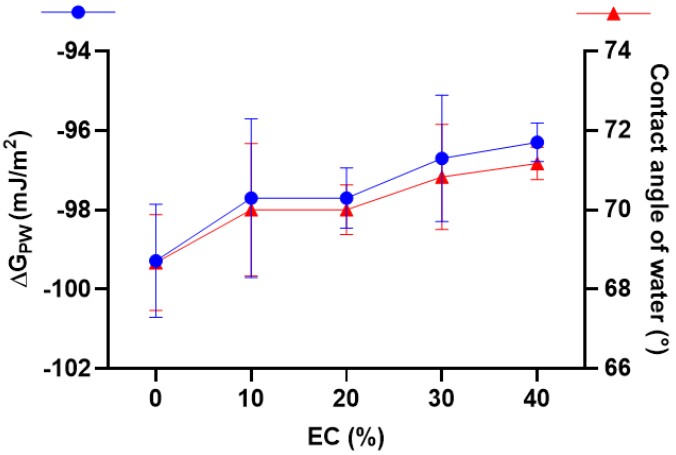


Figure 3.13. The correlation between ΔG_{PW} of combination compacts and their contact angle data of water. (n=6)

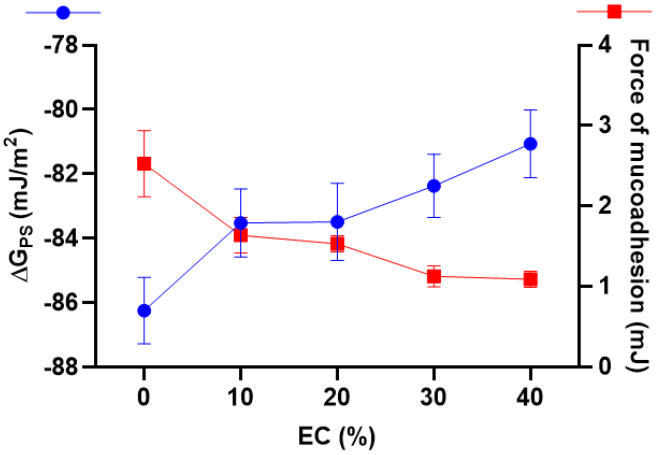


Figure 3.14. The correlation between ΔG_{PS} of combination compacts and their force of mucoadhesion data measured on porcine buccal mucosae. (n=6)

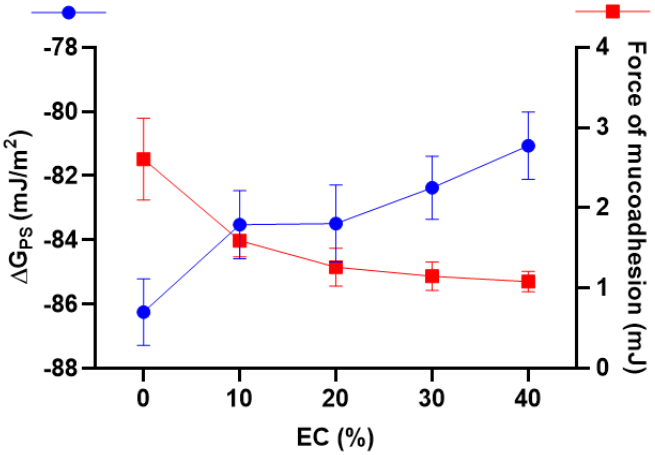


Figure 3.15. The correlation between ΔG_{PS} of combination compacts and their force of mucoadhesion data measured on porcine sublingual mucosae. (n=6)

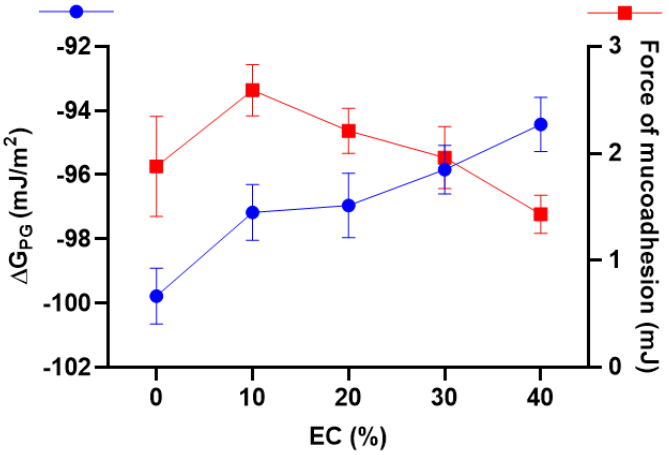


Figure 3.16. The correlation between ΔG_{PG} of combination compacts and force of mucoadhesion measured on porcine stomach mucosae. (n=6)

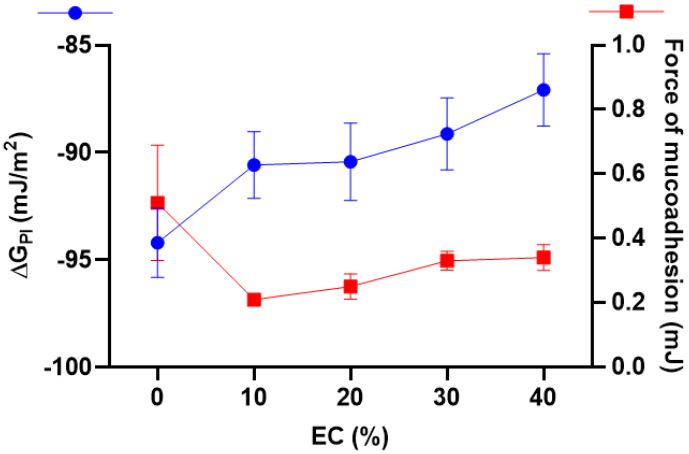


Figure 3.17. The correlation between ΔG_{PI} of combination compacts and their force of mucoadhesion data measured on porcine intestinal mucosae. (n=6)

The total free energy of adhesion ΔG^{TOT} was calculated among combination compacts, mucin and three different fluids (Table 3.11). Interestingly, with the increasing percentage of EC, ΔG^{TOT} decreased. Therefore, when correlated ΔG^{TOT} with force of mucoadhesion, they showed a similar decreasing trend, with the increasing of EC (Figure 3.18-Figure 3.21). A disagreement was found between ΔG^{TOT} and force of mucoadhesion. As discussed in section 3.5.2 that the reduction in mucoadhesive force with increasing percentages of EC was likely attributed to the increased surface hydrophobicity, weakened hydration ability, and decreased entanglement and interpenetration between polymer chains and mucin chains. The decrease in mucoadhesive force was reasonable, thus the disagreement was probably caused by the results of ΔG^{TOT}. Contact angle values were the only experimental data used to conduct the complex calculation of ΔG^{TOT}. Obtaining meaningful contact angle values is extremely difficult, due to the measurement is depending on many experimental conditions such as the surface heterogeneities and asperities, surface cleanliness, and the resolution of measuring equipment and data interpretation. Furthermore, it should be stressed that an ideal solid surface was a critical premise obliged for the application of the Young-Dupre equation in the calculation. However, it is unlikely to have an ideal solid surface in our study, and most of the conditions mentioned above were lacking in control, thus it is rational for the theoretical calculated data to have flaws.

Table 3.11
The Calculated Total Free Energy of Adhesion among Combination Compacts, Mucin, and Different Body Fluids (ΔG^{TOT}, in mJ/m^2)

EC (%)	ΔG^{TOT}		
	Gastric fluid (pH 1.2)	Normal saline (pH 6.4)	Intestinal fluid (pH 7.5)
0	10.14±2.58	13.90±2.58	52.77±3.45
10	11.74±3.01	8.11±3.13	49.72±4.16
20	11.49±1.82	7.66±1.97	49.34±2.67
30	11.19±3.00	7.38±3.06	48.86±4.09
40	9.33±1.72	5.42±1.92	46.27± 2.60

Note. N=6.

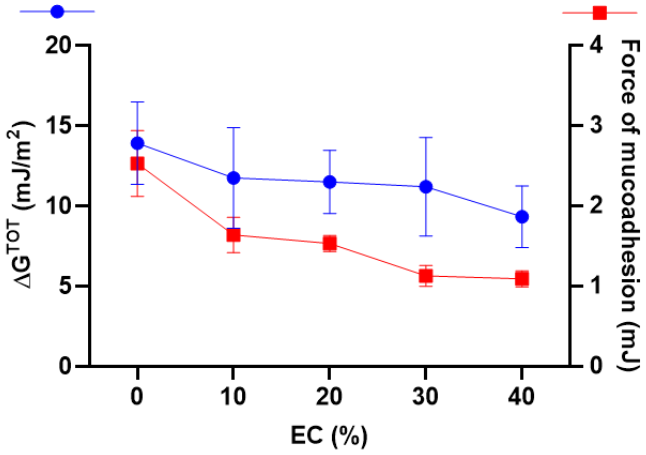


Figure 3.18. The correlation between ΔG^{TOT} of combination compacts and their force of mucoadhesion data measured on porcine buccal mucosae. (n=6)

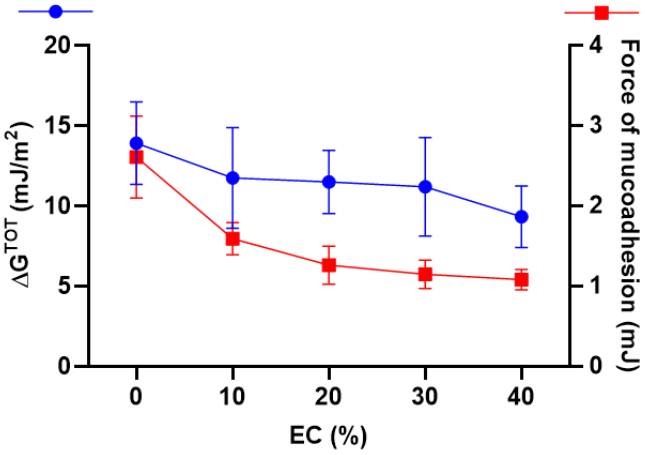


Figure 3.19. The correlation between ΔG^{TOT} of combination compacts and their force of mucoadhesion data measured on porcine sublingual mucosae. (n=6)

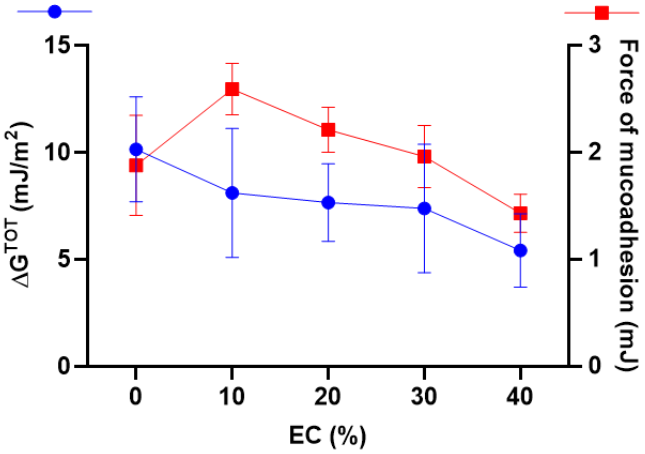


Figure 3.20. The correlation between ΔG^{TOT} of combination compacts and their force of mucoadhesion data measured on porcine stomach mucosae. (n=6)

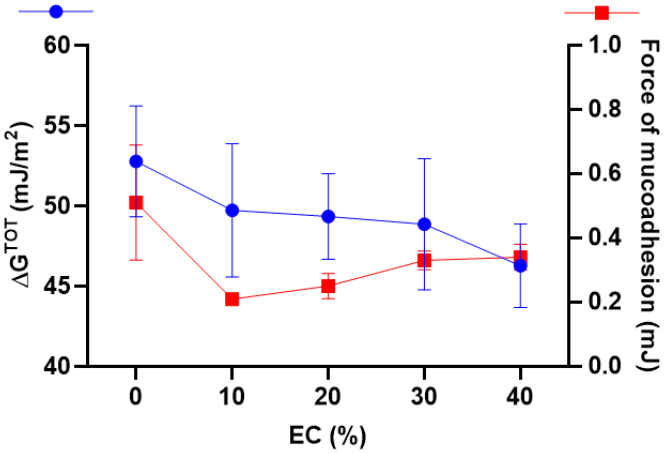


Figure 3.21. The correlation between ΔG^{TOT} of combination compacts and their force of mucoadhesion data measured on porcine intestinal mucosae. (n=6)

3.6. Summary

Thermodynamic analysis of mucoadhesion was conducted on seven grades of HPMC compacts via Lewis acid-base approach for the purpose of predicting the driving force of mucoadhesion. The calculated results of interfacial free energies demonstrated the decrease in surface hydrophilicity with the increasing viscosity of HPMC. The explanation of the decreasing acid-base interaction with increasing viscosity of HPMC was provided by the calculated theoretical number of hydroxyl groups for different grades of HPMC. Moreover, the calculated ΔG^{TOT} showed a disagreement with the measured force of mucoadhesion, which probably due to the boundedness of surface energy analysis and the imperfection in the contact angle measurement.

The combination compacts made up of EC and HPMC grade K15M were prepared for the purpose of modifying the surface hydrophilicity. As expected, the contact angle values of different body fluids were increasing with the increasing percentages of EC involved in the combination compacts. However, the force of mucoadhesion measured on buccal, sublingual

and stomach mucosae showed an overall decline due to the reduced surface wettability. Due to the hydrophobic property and the low viscosity of EC, it was deduced that the weakened hydration ability and the decreased interdiffusion ability between polymer chains and mucin chains might also be the influencing factors contributed to decreased mucoadhesive force. In general, the involving of EC did not improve the mucoadhesive performance.

Furthermore, the same thermodynamic analysis was conducted on combination compacts. The results of interfacial free energy demonstrated the decreasing of surface hydrophilicity of combination compacts with the increasing percentages of EC involved. It is inferred that the surface property of combination compacts regulated the decreasing of mucoadhesive force. However, the calculate ΔG^{TOT} was not accurate possibly due to the experimental defection of contact angle measurement.

CHAPTER 4: CONCLUSIONS

The role of physicochemical properties of mucoadhesive polymers on the performance of *in vitro* mucoadhesion was investigated in this study. HPMC polymer compacts of different commercial grades were selected to investigate their surface hydrophilicity, mucoadhesive force, and hydration behavior. With the increasing viscosity, a decrease in surface hydrophilicity was observed as shown in the increase of contact angle values in different simulated body fluid along with a corresponding increased mucoadhesive force. The positive correlation between contact angle values and force of mucoadhesion was also demonstrated, suggesting that surface property cannot be used alone to describe the mucoadhesive performance.

Due to the ionic property of HPMC, it was insensitive to a range of pH values, which suggested its widely usage in a variety of physiological locations. It was shown that mucosal surface with lower amount of polar lipids might provide a better mucoadhesive performance for HPMC at higher viscosity grades. Therefore, from the formulation point of view and for mucoadhesive purpose, polymers with charges would be a superior choice than non-ionic polymers for sites with relatively greater amount of polar lipids such as intestine.

The hydration ability of HPMC compacts showed an enhancement with their increasing viscosity. According to the dehydration theory, this might imply that the ability of dehydrating the mucus layer was improved, and consequently resulted in higher viscosity grades of HPMC exhibiting higher mucoadhesive force. Compacts with 5 mins pre-hydration showed decreased mucoadhesive force in comparison with dry compacts that may be due to excessively extended polymer chains.

Thermodynamic analysis of mucoadhesion was conducted on HPMC compacts via Lewis acid-base approach for the purpose of predicting the driving force of mucoadhesion. The interfacial free energy between polymer compacts and different media verified the reduced surface hydrophilicity with increasing viscosity. However, ΔG^{TOT} did not show an agreement with the measured force of mucoadhesion, which might be related to the constraint of thermodynamic analysis and the lack of consideration on contact angle hysteresis. Theoretical number of hydroxyl groups was reducing with the increasing viscosity of HPMC, which provided an explanation for the receding acid-base interaction.

EC was involved in the combination compacts with HPMC grade K15M for the purpose of altering the surface property. The addition of EC resulted in decreased surface hydrophilicity and weakened mucoadhesive performance of the combination compacts, which was mostly due to its low surface energy, hydrophobic property, and the low viscosity grade. In the thermodynamic analysis of EC, ΔG^{TOT} also showed a disagreement with the measured force of mucoadhesion. This disagreement may be attributed to the imprecise measurement of contact angles. In general, the addition of EC in the polymer compacts did not result in an improved thermodynamically mucoadhesion process.

Contact angle measurement is an important parameter in the mucoadhesion. Lack of accuracy in its measurement will influence the results on surface properties and the thermodynamic analysis of mucoadhesion in this study. When it comes to a real surface, it is questionable whether it reflects the wettability of that surface by a single contact angle measurement. The behavior of the contact line on a real surface is complex because it depends not only on the implicit wettability of the solid but also other factors. The surface roughness of the polymer compacts was believed as an important factor regulating the outcome of contact

angle in this study, thus further investigations should be conducted to confirm its effect on fluctuating the contact angle values. Moreover, contact angle is in fact a dynamic process rather than static, therefore the measurement should be conducted with the consideration of time. The dynamic measurement of contact angles will potentially produce more reliable results in our research study.

The use of in vitro system to examine the ability of mucoadhesion is certainly helpful for the in vivo performance of formulation [150]. However, the in vitro system is limited of mimicking some of the complex characteristics such as mucus turnover, mucin flow, peristalsis and enzyme secretion inside the human body [151]. Therefore, the results from in vitro experiments may show distinctions with in vivo studies [152, 153]. The use of mucosae from various animal sources other than human may arise further limitations for the predictive capability of these in vitro systems. Moreover, the challenge of selecting the most suitable in vitro systems to decide the rank order of polymer materials concerning their mucoadhesive performance is also encountered [102]. In spite of the difficulties of directly correlate in vitro to in vivo results, it is still achievable under well-established mucoadhesion in vitro test systems [154].

Lastly, in order to have a complete understanding of mucoadhesion mechanism, further investigation on other adhesion theories and the characterization of other polymeric physicochemical properties should be done in future.

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