

Chapter 2

Mucoadhesion and Characterization of Mucoadhesive Properties

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2.1 Introduction

An adhesive is a material that attaches to another substrate surface and resists separation [1]. Adhesion involves the formation of attractive bonds between two substrates that resist separation. Bioadhesion is a specific case of adhesion in which at least one of the two substrates involves a biological tissue [2]. Furthermore, if the adherent substrate surface is a mucosal surface, e.g., a mucosal membrane, bioadhesion is specifically referred to as mucoadhesion [3–5].

The use of mucoadhesive materials for the enhanced delivery of therapeutic agents has been of interest for several years owing to several important advantages concerning the in vitro and in vivo performance of dosage forms. Mucoadhesive formulations are capable of providing localized drug release in desirable regions such as nasal cavity, eye, mouth, stomach, intestine, and vagina to enhance their clinical efficacy. The employment of mucoadhesive materials in formulations may modify the permeability of mucosal tissue or membranes and hence facilitate the adsorption of macromolecules, e.g., peptides. Furthermore, the interaction between mucoadhesive formulations and mucosal surface offers potential to prolong the residence time of the dosage form at the site of application, thereby reducing the dosing frequency and increasing patient compliance [3, 5–8].

Since the first report of the first application of mucoadhesive systems by Scrivener and Schantz [9], there have been many publications regarding the design, development, and testing of bioadhesive and mucoadhesive platforms. Examples of reported mucoadhesive drug delivery dosage forms include tablets, films, gels, creams, ointments, viscous solutions, micro- and nanoparticulate suspensions, and sprays [8, 10]. Commercially, one of the earliest mucoadhesive products was Orabase®, which

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consists of natural gums to facilitate mucoadhesion. So far, a number of mucoadhesive products have been commercialized, e.g., Replens[®], Zidoval[®] gels, for vaginal therapies.

2.2 Structure, Composition, and Functions of Mucosa, Mucus, and Mucin

Mucoadhesive formulations are designed to form specific interactions with mucin-coated mucosal membranes which, typically, are composed of specialized epithelium, lamina propria, and glands (depending upon their type and location) [11–13]. The mucosal membrane covers the epithelium and facilitates the exchange of gases and nutrients between the underlying epithelium and the external environment. In addition, such membranes inherently lubricate cavities and passages and form a barrier to protect the epithelium from damage associated with pathogens and noxious substances [11, 14–15]. Mucus which is secreted by goblet cells within mucosal membranes is the most crucial component responsible for mucosal protective functions as well as adsorption and exchange of other components, notably drugs [15, 16]. Structurally, mucus is a complex viscous gel that is primarily composed of water (circa 95 %) and mucin (a glycoprotein), electrolytes, fatty acids, phospholipids, cholesterol, proteins, and other various species in smaller proportions [5, 11]. The presence of mucin on the epithelia of many cavities including the mouth, nose, eyes, vagina, rectum, and the stomach has been confirmed. Furthermore, it has been shown that there are several types of mucins in vaginal fluid [17–18], saliva [19], tears [20], and within the gastrointestinal tract [21]. The mucin glycoproteins exhibit a highly entangled network of macromolecules that associate with one another through non-covalent bonds. Such molecular association is central to the structure of mucus and is responsible for its rheological properties [5, 22–27]. Mucin can be considered as an anionic polyelectrolyte at neutral pH owing to the presence of pendant sialic acid and sulfate groups located on the glycoprotein molecules through covalent bonds [27]. These acidic groups exhibit pKa values from 1.0 to 2.6 resulting in their complete ionization under physiological conditions [8, 28]. The negative charge of mucin has been reported to be important in partitioning and complex formation with pharmaceutical preparations [27, 29]. Mucin is also fundamental to cytoprotection; the endothelial and leukocyte classes of mucins being adhesion molecules that are involved in lymphocyte homing and lymphocyte activation or are part of the adhesion cascade that plays a role in the initiation of inflammation [30–31]. A thorough understanding of mucin is fundamental to the potential pharmaceutical applications of mucoadhesive dosage forms.

Mucins are macromolecules with the molecular weight range from 0.5 up to 20 MDa. The basic composition of a typical mucin includes about 80 % carbohydrates, namely *N*-acetylglactosamine, *N*-acetylglucosamine, fucose, galactose, sialic acid, and traces of mannose and sulfate, and 20 % protein core [8, 15]. The central protein

segments are composed of a large number of tandem repeats rich in serine, threonine, and proline that are all linked by the interspersed regions possessing little *O*-glycosylation, a few *N*-glycosylation sites, and a high proportion of cysteine domains (> 10 %) [32]. In addition, it has been shown that large carbohydrate side chains link to the hydroxyl side chains of serine and threonines of the protein core via *O*-glycosidic covalent bonds [15]. The terminal regions of an entire mucin chain where little glycosylation occurs are referred to as “naked protein regions” [5, 33].

Although mucin has been reported to be difficult to characterize due to the large molecular weight, polydispersity, and high degree of glycosylation, the significant interest and progress in research have been focused on identifying and distinguishing mucin genes. There have been at least 19 mucin sequenced by cDNA cloning, and three of them have been totally sequenced, namely MUC1, MUC2, and MUC5B [15, 34]. Other techniques employed to characterize mucin include light scattering [35–39], nuclear magnetic resonance (NMR) [40–43], transmission electron micrographic (TEM) [30, 44], and atomic force microscopy (AFM) [45–48].

2.3 Mucoadhesion Theories of Polymer Attachment

Mucoadhesion is a complex process and has not yet been fully understood [8, 26]. Several theories have been proposed to explain mucoadhesion, notably: (1) the wetting theory [49–52], (2) the mechanical interlocking theory [1], (3) the electronic transfer theory [1], (4) the diffusion-interpenetration theory [50], (5) the adsorption theory [53], and (6) the fracture theory [53]. They are briefly detailed below.

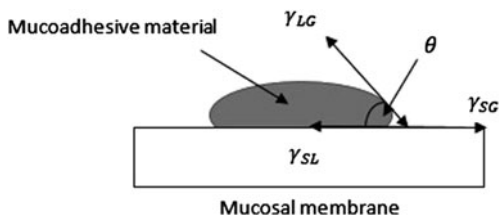
2.3.1 Wetting Theory

The wetting theory attributes the bonding between the formulation and the surface tissue to intermolecular interaction and interfacial tension. This theory is usually applied for liquid or low viscosity mucoadhesive systems and is essentially a measure of the “spreadability” of a drug delivery system across the biological substrate [5, 54]. The spreadability of the system is indicative of interactions and can be measured by the liquid–solid contact angle. Adhesive forces between a liquid and solid enable a liquid drop to spread across the surface, whereas, cohesive forces within the liquid cause the drop to ball up and avoid contact with the surface. Generally, contact angles less than 90° indicate that the wetting of the surface is favorable, and the liquid tends to spread out to a large area. A contact angle greater than 90° indicates the wetting of the surface is unfavorable; the interaction among liquid molecules maintains the shape of the droplet and minimizes its contact area to the solid surface [55].

The contact angle may be experimentally measured from which interfacial tension (γ) may be derived using the Young equation [49, 56]:

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cos \theta \quad (2.1)$$

Fig. 2.1 Contact angle measurement between a droplet and solid surface. (Modified from [5])



Where γ_{SG} is the interfacial tension between solid and gas; γ_{SL} is the interfacial tension between solid and liquid; γ_{LG} is the interfacial tension; and θ is the contact angle between solid and liquid interface (Fig. 2.1).

The interfacial tension associated with contact angle θ exhibits the degree of wetting. When the contact angle θ is 0° , wetting is complete, the liquid having fully spread across the surface of the substrate. In contrast, a contact angle of 180° is indicative of nonwettability. Wetting between the liquid (formulation) and the substrate (e.g., mucus) substance occurs whenever the contact angle ranges between 0° and 180° [54, 57].

2.3.2 Mechanical Interlocking Theory

The mechanical interlocking theory only considers the adhesion between liquid and a rough surface or a surface rich in pores [58–60] and essentially proposes that the adhesion between the two substrates is due to mechanical interlocking of the adhesive into the irregularities of the substrate surface [1]. Adhesion between the mucoadhesive system and the rough surface typically occurs within a diverse biological environment and accordingly this theory does not fully explain the adhesive properties in vivo [1, 60–61].

2.3.3 Electronic Transfer Theory

In the electronic transfer theory, mucoadhesion occurs as the result of the transfer of electrons between mucus and the mucoadhesive platform. The electronic transfer between two different layers results in the formation of a double-layered electronic charge at the interface. This theory suggests that the electrostatic forces are critical in generating bond adhesions rather than high joint strength [1, 60, 62–65].

2.3.4 Adsorption Theory

There are various surface interactions that result in adhesion, including primary bond and secondary bond formation. In the former situation, primary bonds (e.g.,

ionic and covalent bonds) are undesirable because they form a strong energy barrier, which may result in permanent interactions with mucus or tissue layer [1]. In contrast, secondary (weaker) bonds such as van der Waals forces, hydrogen bonding, electrostatic attraction, and hydrophobic interactions are more desirable, resulting in semipermanent interactions (an important criterion for drug delivery systems) [1, 5, 53, 66].

2.3.5 Fracture Theory

According to the fracture theory, the adhesive strength, also known as the fracture strength, is related to the force required to separate the platform and the mucus surfaces. Previous publications have suggested the use of Young's modulus of elasticity (E), the fracture energy, (ε) and the critical crack length (L) to determine the fracture strength (σ). The equation is shown below [52]:

$$\sigma = \left(\frac{E \times \varepsilon}{L} \right)^{1/2} \quad (2.2)$$

The fracture energy can be obtained from the reversible adhesive work:

$$\varepsilon = \varepsilon_r + \varepsilon_d \quad (2.3)$$

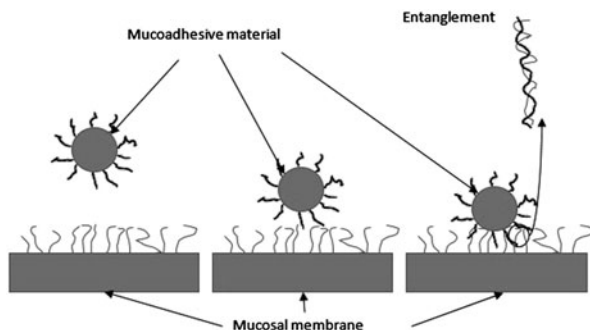
where ε_r is the required energy for producing new fractured surfaces and ε_d is the work of plastic deformation provoked by the removal of a proof tip until the disruption of the adhesive bond.

Depending on the position of their occurrences, fractures may be divided into platform fracture, platform-mucus fracture, and mucus fracture. As a result, the fracture theory is not only used to measure adhesive strength between the platform surface and the mucus surface, but can also be used to evaluate the strength of intermolecular interactions within the platform.

2.3.6 Diffusion-Interpenetration Theory

The diffusion-interpenetration theory is a commonly employed theory to describe mucoadhesion and involves the interpenetration and entanglement between the polymer chains and the mucus chains [67]. The first step in this process involves the creation of an initial contact between the bioadhesive polymer chains and the mucus chains. In this step, weak physical forces, e.g., attraction and electronic force, dominate the mobility of the polymer chains. The second step involves the interpenetration of polymer chains from the delivery system into mucus layer to achieve mucoadhesion via more substantial bond formation. The profile of the

Fig. 2.2 Schematic profile of interpenetration steps. (1) Polymer chain approaching the mucus layer. (2) Interpenetration of polymer into mucus chains. (3) Polymer chains and mucus layer contact by physical-chemical forces



diffusion-interpenetration theory is shown in Fig. 2.2. The depth of interpenetration is dependent upon the diffusion coefficient of both polymer and substrate, time of contact, and the adhesive strength of the bioadhesive polymer [4, 5, 60, 61, 68, 69]. Mikos and Peppas introduced the relationship between interpenetration depth and characteristic time:

$$\tau = l^2/D_b \quad (2.4)$$

Where l is the interpenetration depth and D_b the bioadhesive diffusion coefficient through mucus [50].

For significant interpenetration to occur, diffusion of the polymer chains of the dosage form into the mucin layer (and vice versa) must occur. Furthermore, the two components should have similar chemical structure to obtain the strongest mucoadhesive interaction [64].

Although the theories that have been described in this section may be helpful in describing the mucoadhesive behavior between polymer and mucus theoretically, the real situation is more complex to be explained or modeled using a single theory. Thus, a combination of two or more theories is always employed to characterize the complex phenomenon [4, 60].

2.4 Mucoadhesive Polymers

The possibility of mucoadhesion and the interaction strength can be influenced by the polymer structural and functional groups [5]. At present, the most commonly used mucoadhesive polymers are composed of polar chemical functional groups such as hydroxyl ($-\text{OH}$), carboxyl ($-\text{COOH}$), amide ($-\text{NH}_2$), and sulfate ($-\text{SO}_4\text{H}$) groups that are able to interact with the mucin glycoproteins [5, 60, 70]. The interactions between polymers and mucin include physical entanglements and secondary interactions notably hydrogen bonds [71]. The contributions from such forces facilitate the formation of a strengthened cross-linked network and hence achieve mucoadhesion [27].

Table 2.1 Commonly used adhesive polymers. (Modified from [118])

Type	Common polymers
Anionic polymers	Carbopol® Polycarbophil® Sodium alginate Sodium carboxymethylcellulose
Cationic polymers	Chitosan
Nonionic polymers	Hydroxypropylmethylcellulose Hydroxypropylcellulose Methylcellulose Polyethylene glycol Polyvinylpyrrolidone Hydroxyethylcellulose
Stimuli-sensitive polymers	Poloxamer

Mucoadhesive polymers can be classified according to the chemical characterization of the polar functional groups as shown in Table 2.1. The applications of these polymers as mucoadhesive drug delivery platforms is described in other chapters in this book and will not be addressed in this chapter.

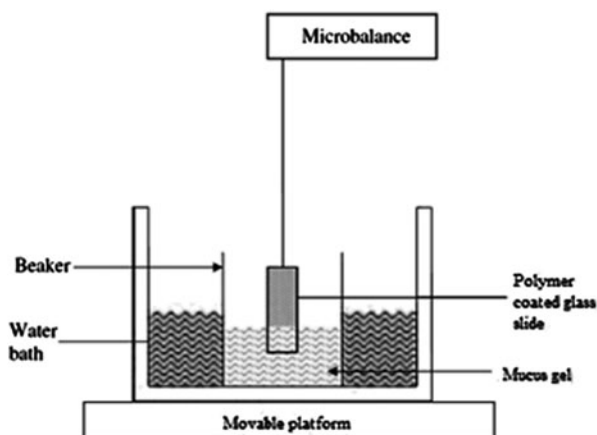
2.5 Techniques Utilized for the Assessment of Mucoadhesive Strength

Despite the accumulation of numerous studies concerning the in vitro and in vivo performance of mucoadhesive drug delivery systems, surprisingly, there has not been a standard technique designed for mucoadhesive measurement or any analytical method that can be employed to qualify mucoadhesive strength. The lack of a uniform method to assess retention at the site of application may compromise the selection of formulations for clinical examination. At present, researchers have developed several approaches to rank the mucoadhesive properties of polymers and formulations and to understand their adhesive behavior. The developed techniques can be categorized as in vitro or in vivo methods.

2.5.1 *In Vitro Techniques Used for Mucoadhesion Characterization*

In vitro tests are the most common and convenient methods to assess the mucoadhesive properties of candidate formulations [72]. These techniques typically assess mucoadhesion using tensile force measurements that assess forces of attachment and detachment, and/or flowing techniques that evaluate the influence of shear stress, and measurement of the residence time of a mucoadhesive formulation on mucosal membrane. These methods have evolved from simple analytical techniques to more sophisticated and comprehensive procedures.

Fig. 2.3 The apparatus for the modified Wilhelmy plate technique. (Modified from [5])



Tensile Force Measurements There have been several reports of methods that have been used to characterize mucoadhesion based on the measurement of tensile force to break the interaction between the mucoadhesive drug delivery platform and the test substrate. Examples of these are detailed below.

Tensile measurement using microbalance (the modified Wilhelmy plate technique)

This method was first employed to determine the detachment force by Smart and coworkers in 1984 [73]. The method is based on the Wilhelmy plate method and consists of a glass plate and a microforce balance (Fig. 2.3). In their work, the glass plate was coated by dipping into a 1 % solution of the test material, and subsequently drying the moisture at 60 °C in an oven to constant weight. The glass plate was hung on to a microforce balance and in contact with 1 ml homogenized mucus contained in a 5 ml glass vial which was placed on a vertical movable platform. The platform was raised until the plate had penetrated the mucus gel or model material to touch the base of the container. The platform was lowered subsequently following 7 min contacting of mucus gel and sample material while the maximum force for detachment was recorded by the microforce balance. To make a standard experiment, the detachment force between mucus gel and a clean glass plate was tested, and the coated plate force was then expressed as a percentage of the clean plate force. In their work, several materials have been tested to obtain the mucoadhesiveness. Those materials were ranked according to the strength of mucoadhesion and the rank order agreed with that published by Chen and Cyr [74]. Furthermore, the effects of contact time, molecular weight, and pH value on adhesion were studied. The mucoadhesive forces of several materials are listed in Table 2.2.

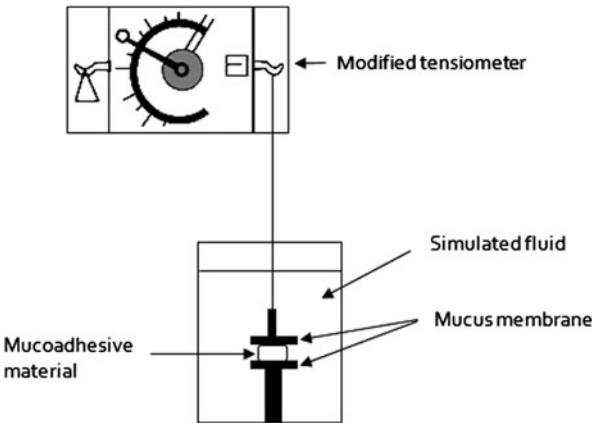
This modified Wilhelmy plate technique was probably the first method employed to screen the potential of polymers as mucoadhesive platforms for use as buccal films [75]. It is a simple and efficient method that provides information about possible mucoadhesive strength; however, it may be limited due to the dissolution of polymer candidates in mucus gel and the absence of biological tissue [50, 75].

Table 2.2 Rank order of mucoadhesive force. (Modified from [74])

Coating material	Mean adhesive force (%)	Standard deviation
75P SCMC	192.5	12.0
Carbopol 937	185.0	10.3
Tragacanth	154.4	7.5
Gantrez AN	147.7	9.7
Sodium alginate (H.V.)	126.2	12.0
Hypromellose (M.V.)	125.2	4.8
Gelatin	115.8	5.6
Pectin	100.0	2.4
P.V.P	97.6	3.9
Acacia	97.6	5.9
PEG 6000	96.0	7.6

H.V. high viscosity, M.V. medium viscosity

Fig. 2.4 A modified surface tensiometer for measurement of detachment force of mucoadhesion. (Modified from [75])



Techniques based on modified tensiometers and advanced dual tensiometers These techniques are modifications of the Wilhelmy plate technique [76]. As shown in Fig. 2.4, a mucoadhesive material is placed between two tissues, with the upper tissue suspended from a tensiometer spring to record forces, and the lower tissue fixed on a weighed glass vial in a beaker containing simulated fluid. The upper tissue is subsequently raised following a period of contact between the mucoadhesive material and tissues. A detachment force is recorded by the tensiometers to present the maximum loading that the mucoadhesive interaction could withstand prior to separation [77].

More recently, Abruzzo and coauthors [78] presented an alternative means to characterize chitosan/gelatin films. In their study, the *in vitro* and *in vivo* mucoadhesion characterizations were conducted using the modified surface tensiometer method in five healthy volunteers aged 25–40 years, respectively. They demonstrated that the *in vivo* residence time of the film in the buccal cavity was related to the *in vitro* mucoadhesive strength, notably detachment force.

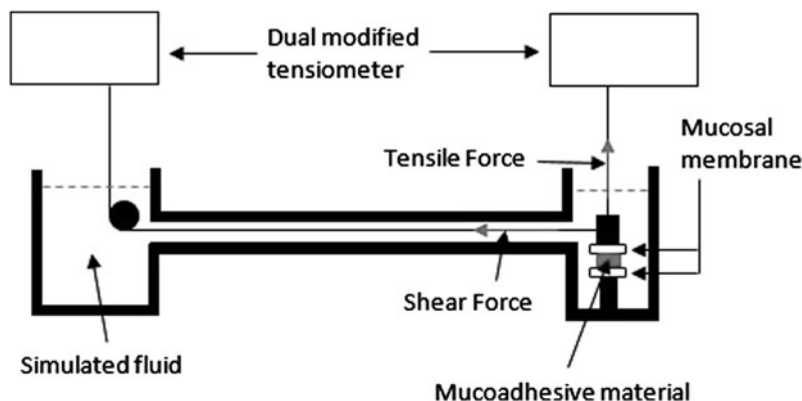


Fig. 2.5 Dual tensiometer apparatus. (Modified from [3])

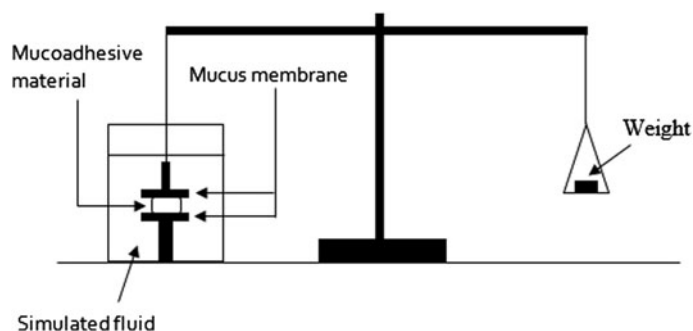


Fig. 2.6 Modified physical balance used to measure mucoadhesive strength. (Modified from [79])

In a further modification, the dual tensiometer method has been designed to characterize the effect of shear stress on mucoadhesion. The apparatus was constructed using two tensiometers that measured tensile stress and shear stress, respectively. The second tensiometer was connected to a standard single tensiometer apparatus to stretch the upper tissue from the left or right side (Fig. 2.5). A shear stress is recorded on the second tensiometer to indicate the strength of mucoadhesion in the horizontal direction.

Tensile force measurement using a balance A series of methods to assess the mucoadhesive properties of materials using a modified physical balance have been described by Gupta [79]. As shown in Fig. 2.6, this consists of a balance with two arms on which weights are suspended, the weight corresponding to the detachment force being determined.

More specifically, in this method the formulation is located between the two tissue layers in a glass beaker containing a defined amount of fluid. Weights are gradually added to one arm of the balance; the fracture of the mucus/sample interface being

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