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Review Article

# Pharmaceutical applications of mucoadhesion for the non-oral routes

Katarina Edsman and Helene Hägerström

## Abstract

The adhesion of pharmaceutical formulations to the mucosal tissue offers the possibility of creating an intimate and prolonged contact at the site of administration. This prolonged residence time can result in enhanced absorption and, in combination with a controlled release of the drug, also improved patient compliance by reducing the frequency of administration. During the almost 30 years over which mucoadhesion has been studied, a considerable amount of knowledge has been gained, and much has been learned about the different mechanisms occurring at the formulation–mucus interface and the properties that affect these mechanisms. The in-vivo performance of a dosage form not only depends on the mechanisms occurring at the interface, but also on the properties of the total mucoadhesive complex: the dosage form, the mucosa and the interface between them. A wide variety of methods are used for studying mucoadhesion; some rather similar to the in-vivo situation and some mimicking the interface alone. In this review, the mucus surface, the methods used for the study of mucoadhesion, the different mechanisms involved in mucoadhesion and theories underpinning them have been described. The complexity of mucoadhesion when trying to systemize the subject will also be discussed. The last part of the review describes the buccal, nasal, ocular, vaginal and rectal routes and provides examples of what can be achieved in-vivo when using mucoadhesive formulations.

## Introduction

The term bioadhesion is commonly defined as adhesion between two materials where at least one of the materials is of biological origin. In the case of bioadhesive drug delivery systems, bioadhesion often refers to the adhesion between the excipients of the formulation (i.e. the inactive media) and the biological tissue. The term mucoadhesion can be considered to refer to a subgroup of bioadhesion and, more specifically, to the case when the formulation interacts with the mucus layer that covers a mucosal tissue. In practice, however, these two terms are often used interchangeably.

The term bioadhesion has been used in the literature for several decades. When searching the databases Chemical Abstracts and Medline for the term bioadhesion, it was found that the term first appears in a review on adhesion published in 1968 (Baier et al 1968). Earlier, the term biological adhesion had been used in studies concerning cell adhesion to different materials. In the 1970s several studies were published, mainly in the area of biomaterials research, where bioadhesion and biocompatibility are important issues. The idea of using bioadhesive materials in the development of pharmaceutical formulations appeared in scientific articles in the early 1980s. The aim was to develop drug delivery systems that would increase the absorption of a drug, for both local and systemic effects, as a result of intimate and prolonged contact at the site of absorption. Among the early work on bioadhesive systems is that of Nagai and coworkers, who showed that the treatment was improved for several administration routes when adhesive formulations were used (Nagai 1985). For example, the treatment of aphthae, an infection in the mouth, and the treatment of uterine cancer were improved with local delivery using mucoadhesive tablets. In addition, mucoadhesive preparations for delivery of insulin through the buccal and the nasal routes of administration were investigated.

The term mucoadhesion appeared in the literature for the first time in 1977 in a medical research paper describing a clinical trial of a locally delivered anaesthetic

Dept of Pharmacy, Uppsala University, Uppsala Biomedical Centre, P.O. Box 580, SE-751 23 Uppsala, Sweden

Katarina Edsman, Helene Hägerström

**Correspondence:** K. Edsman, Dept of Pharmacy, Uppsala University, Uppsala Biomedical Centre, P.O. Box 580, SE-751 23 Uppsala, Sweden. E-mail: katarina.edsman@farmaci.uu.se

(Goldstein et al 1977). In the mid and late 1980s the concept of mucoadhesion became more commonly recognised. Over the years, mucoadhesive and bioadhesive systems have been used for nasal, ocular, buccal, vaginal, rectal and oral drug delivery.

In early studies of mucoadhesion, different methods were developed or modified from other areas of adhesion research. The majority of the methods were based on an in-vivo-like situation, usually measuring the contact time or the force required to separate the formulation from the tissue (Ishida et al 1983; Gurny et al 1984; Smart et al 1984). In parallel with the development of methods, different theories were proposed, including some adapted from traditional adhesion theories. Most of the early work on bioadhesive polymers was performed with commercially available polymers, often in the form of powders (Ch'ng et al 1985; Park & Robinson 1985), tablets (Ponchel et al 1987), coated spheres (Teng & Ho 1987) or dried films (Smart et al 1984). From these kinds of studies, general conclusions were drawn about some of the physicochemical characteristics of a good bioadhesive material, such as molecular weight, cross-linking density and charge.

Nowadays, mucoadhesion figures in the literature, covering a wide variety of applications. In some studies, the term mucoadhesive formulation is used in a routine and noncritical way, e.g. different formulations and polymers have been ranked as more or less mucoadhesive by using randomly chosen methods. On the other hand, several studies have been made so as to really understand mucoadhesion. New methods and polymers have been developed and used with the intention of learning more about the kind of interactions that can occur between the formulation and the mucosa. But even now, the understanding of the phenomenon is not yet complete. One of the reasons for this is probably that there are so many different formulations involving a large variety of adhesion mechanisms that no single existing theory can explain them all.

In this review, the aim is to present the different theories and methods that have been used, and to discuss the different mechanisms involved in adhesion. In the last section, the most common routes of administration will be presented along with examples of mucoadhesive formulations used.

## The mucosal surface

The mucosa or the mucous membrane is the moist tissue that lines organs and body cavities such as the mouth, gut, nose and lungs. The mucosa consists of the epithelium itself and the supporting loose connective tissue, called the lamina propria, immediately beneath the epithelium. Deeper connective tissue, which supports the mucosa, is called the submucosa. The epithelial layer can either be a single layer, as in the intestine and the bronchi, or a multilayered epithelium, as in the vagina, the mouth and the cornea. In single-layered epithelia there are non-specialized and specialized (goblet cells) epithelial cells that secrete mucus directly onto the surface of the epithelium. The multilayered stratified epithelium contains, or is adjacent to, tissue that contains specialized glands (e.g. salivary glands) secreting mucus. The main function of the mucus gel is to mediate the interactions between the epithelial cells and their environment via such processes as lubrication, maintaining the water balance and binding particles, bacteria and viruses, and it may also play a role in the immune response.

The main constituents of the mucus gel are glycoproteins, lipids, water and electrolytes; the water content is approximately 95%; the glycoproteins and lipids constitute between 0.5 and 5%; and about 0.5–1% is composed of mineral salts and approximately 1% free proteins. The exact composition may vary depending on the origin and the role of the mucus and on the health status of the individual in question.

The mucins are a family of glycoproteins with a molecular weight of 1–40 million Da. They are found in two forms – soluble secretory mucin and membrane bound mucin. Secretory mucins form viscoelastic gels because of their high molecular weights and their ability to form complexes as a result of intermolecular disulfide bridges and hydrophobic interactions. The core of the molecule is a protein, usually with a high serine and threonine content, with hundreds of O-glycosidic-linked oligosaccharides bound to it in a "bottle-brush" arrangement (Figure 1). The oligosaccharides constitute approximately 50–80% of the dry weight of the mucins and are responsible for giving an extended conformation to the mucins. The variation in the monosaccharide composition, branching, etc., of the oligosaccharides is large, in addition to which, they vary in length from one to twenty sugars and can be neutral, sialylated or sulfated.

There has been much progress in mucin research during recent years, especially in identifying and sequencing the genes responsible for the different types of mucins. For a more detailed description of the properties of the mucins, see, e.g. Carlstedt et al 1985; Roussel et al 1988; Bansil et al 1995; Campbell 1999; Dekker et al 2002 and references therein.

## Mechanisms and theories of mucoadhesion

Adhesion was studied long before the interest in the bioadhesion and mucoadhesion of pharmaceutical formulations began. Thus, the theories that were developed to understand and explain the adhesive performance of adhesives, paint, glues, etc., have been adapted to gain an understanding of mucoadhesion and bioadhesion. The five theories that are most commonly presented in conjunction with bioadhesion are the absorption, diffusion, electronic, fracture and wetting

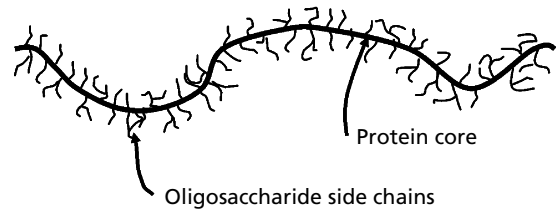


**Figure 1** Schematic structure of a mucin molecule.

**Table 1** Theories of bio-/muco-adhesion

| | |
|---|---|
| The adsorption theory | According to this theory, the formulation adheres to the mucosa as a result of secondary chemical bonds, such as van der Waals forces, hydrophobic interactions, electrostatic attractions and hydrogen bonds. |
| The diffusion theory | In the diffusion theory, the polymer chains of the formulation diffuse into the mucus network and vice versa. A semi-permanent adhesive bond is formed through entanglements of the chains in the interpenetration layer. |
| The electronic theory | The electronic theory assumes that an electron transfer develops from the contact between the polymer of the formulation and the mucus as a result of differences in their electronic structure. This leads to the formation of an electrical double layer at the interface. Adhesion occurs because of attractive forces across the double layer. |
| The fracture theory | The fracture theory is related to the separation of two surfaces after adhesion, and the fracture strength is regarded as being equal to the adhesive strength. It assumes that the fracture occurs exactly at the interface, which is rare or nonexistent. It is mainly used for calculation of adhesive bonds for rigid formulations. |
| The wetting theory | This theory was developed for liquid preparations using the interfacial tension to predict spreading and adhesion. From the measured surface and interfacial tension the work done in an adhesive bond can be calculated. |

theories. These are briefly summarised in Table 1; for a more detailed description of the theories, see, e.g., the following reviews and references therein: Peppas & Buri 1985; Jimenezcastellanos et al 1993; Gandhi & Robinson 1994; Chickering & Mathiowitz 1999; Lee et al 2000.

None of these theories can explain mucoadhesion on its own for all of the different pharmaceutical formulations, but several of these theories can be combined to obtain a picture of the mucoadhesion process. Depending on the formulation (e.g. whether or not the dosage form is hydrated), some theories are more applicable than others, but the relevance of the various theories is also dependent on the thickness of the mucus layer.

A few mechanisms, though, are usually used to describe the processes that occur at the interface between the dosage form and the mucosal tissue. The first step in the mucoadhesion process is the creation of intimate contact between the dosage form and the mucosa. In the case of semisolid or liquid dosage forms, the intimate contact is believed to occur as a result of wetting and of the spreading of the dosage form, which increases the area of contact. For dry and not fully hydrated dosage forms, the wetting, hydration and swelling of the dosage form will initiate the intimate contact with the tissue. Secondly, there will be an interpenetration of the components, the polymers of the dosage form and the mucus gel network on the surface of the tissue, across the interface. The interpenetrated chains can then interact, resulting in entanglements and weak chemical bonds, originating from electrostatic attraction, hydrophobic interactions, van der Waals forces and hydrogen bonds.

Several attempts have been made to prove that this interpenetration layer does indeed exist. In a study by Lehr et al (1992b), electron microscopy was unable to prove its existence in the micrometre range, although the authors could not exclude interpenetration in the nanometre range. Slightly later, however, attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR) was used to show that mucin from a solution deposited onto a cross-linked poly(acrylic acid) (PAA) film interpenetrated with the polymer at the PAA–mucin interface (Jabbari et al 1993). It was subsequently shown using confocal laser scanning microscopy that fluorescent-labelled PAA with a molecular weight of 2–3000 kDa could interpenetrate the mucus gel layer of porcine intestinal mucosa (Imam et al 2003). The measurements that were conducted revealed that there was an inverse correlation between the amount of PAA involved in the interpenetration and the molecular weight – the higher the molecular weight, the lower the degree of interpenetration.

The existence of interpenetration of the polymer and mucin chains has been questioned and debated, and an alternative explanation for the strengthening of the interface has been proposed (Smart 1999). In this theory, it is suggested that dry and partially hydrated dosage forms swell, thereby dehydrating the mucus gel, and that it is this movement of water, rather than interpenetration, that drives the consolidation of the adhesive joint.

## The mucoadhesive complex and removal mechanisms

When a formulation adheres to the mucosal tissue, there are at least three layers (Figure 2) – the dosage form, the mucosa and the interfacial region – that are important for the length of contact between the dosage form and the tissue, i.e. the residence time of the formulation. When a failure occurs, it takes place in the weakest of these regions. Which region is the weakest will depend on the dosage form and the status of the mucus layer. For liquid and semisolid vehicles, the vehicle itself may very well be the weakest

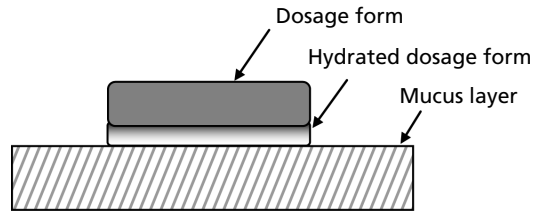


**Figure 2** The mucoadhesive complex.

region (Hagerstrom & Edsman 2001; Hagerstrom et al 2004) because of the weak cohesive forces within the vehicle or because the cohesive forces become weak as a result of dilution by body liquids. For solid dosage forms, the cohesive forces keeping the dosage form together are normally rather strong, so then the failure can either occur in the mucus layer or in the interface region. For solid dosage forms that hydrate after being administered, the hydrating layer of the dosage form often becomes the weakest region (Mortazavi & Smart 1994b).

Dry or partially hydrated polymers swell when in contact with water as a result of osmotic forces. For a cross-linked polymer, there is a maximal equilibrium swelling in water that is determined by its degree of cross-linking; for non-cross-linked polymers, the equilibrium state is a solution. For two gels in contact (i.e. the formulation and the mucus gel) there is a flow of solvent between the gels until the chemical potential has reached an equilibrium. Water transport from the tissue to the formulation affects the residence time in two opposing ways: it will weaken the formulation by hydration/dilution, but it will also strengthen the mucus layer through dehydration of the mucus gel. In a study of the water uptake as measured by the weight gain of compacts and gels in contact with mucus (Mortazavi & Smart 1993), it was found that the weight gain started rapidly and was proportional to the concentration of the gel-forming compound. The properties of the mucus gel were also studied, revealing that the adhesive and cohesive nature of the mucus gel increased as the water content decreased. Nuclear magnetic resonance (NMR)-microscopy has been used to study the diffusion coefficients of the water in the mucoadhesive complex for dry and prehydrated alginate matrices (Marshall et al 2001). A decrease in the diffusion coefficient of water was demonstrated in the mucus layer adjacent to the matrix after 1 min, extending further into the mucus layer at longer times. Using confocal fluorescence microscopy, the authors also showed that there was an increase in the mucus concentration, corresponding to dehydration.

The rate of swelling also affects the duration of adhesion (Mortazavi & Smart 1994b), with faster swelling resulting in adhesion of shorter duration. For dry and partially hydrated dosage forms, swelling studies are often performed in parallel with mucoadhesion studies (NguyenXuan et al 1996; Shojaei & Li 1997; Rossi et al 1999a; Eouani et al 2001; Leitner et al 2003a; Roldo et al 2004) to obtain more information. Mortazavi & Smart (1994b) suggested that the ideal candidate for long-term mucoadhesion is a dosage form that rapidly forms strong interactions with the mucosa, but only allows limited hydration to form a rigid gel. It has also been shown that when hydration of the dosage form plays an important role, the relative adhesive strength is similar if the dosage form is applied on a mucosal tissue or on other surfaces, such as poly(vinyl chloride) (PVC) tape or plexiglass (Mortazavi & Smart 1995; Jacques & Buri 1997).

Similarly, in nonpolymeric dosage forms, the water transport can be one of the mechanisms behind mucoadhesion. Lipid dosage forms based on glyceryl monooleate (GMO) and glyceryl monolinoleate (GML), for example, have been shown to have mucoadhesive properties (Nielsen et al 1998). The mechanism behind this mucoadhesion was unspecific, but it was suggested that it might involve dehydration of the mucosa. The water uptake has an effect on mucoadhesion, as measured by the detachment force, for liquid crystalline phases from GMO (Lee et al 2001). A higher water concentration in the liquid crystalline phase led to a more ordered structure with weaker mucoadhesion. For strong mucoadhesion to occur, the liquid crystalline phase should contain a minimal amount of water. The lamellar phase, which contains less water, was more mucoadhesive than the cubic phase, and, furthermore, more concentrated lamellar phases were more mucoadhesive than lamellar phases with a higher water content.

## Methods used for mucoadhesion studies

There are many methods that have been developed for mucoadhesion measurement. Some are similar to the in-vivo situation and are useful when comparing different materials and formulations to find out which may give the longest residence time. Others have been employed to study the mechanisms of mucoadhesion. The usefulness of the different methods depends on the characteristics of the dosage form and what kind of information is being sought.

The choice of method for studying mucoadhesion is not easy and affects the results obtained. Methods based on measuring the force or work required to detach the formulation from the tissue are quite similar to the in-vivo situation, but may actually give values that are correlated to the cohesion of any of the layers in the mucoadhesive complex (Figure 2). On the other hand, measurements based on a simulation of the interpenetration layer will only be relevant if there is an interpenetrating layer, and the result obtained will only be correlated to the expected performance if the failure actually occurs in this layer. For practical purposes, an analysis of where the failure occurs may not be important, but if we want to learn how to optimize dosage forms and to develop new dosage forms, a deeper understanding of the mechanisms involved is essential. Everyone working in the field of mucoadhesion will have to make a decision about whether the mechanisms are important for the work they are undertaking, or if the aim is to make a measurement that will correlate with the in-vivo situation, and then an appropriate method must be chosen.

When using in-vitro methods, not only the method must be chosen, but also the mucus substrate. It could either be excised tissue or a mucus preparation. An excised tissue has the advantage of providing an in-vivo-like situation, where the surface with which the formulation will interact is as similar to the in-vivo situation as possible. There will be variations in the results depending on the source of the mucosa (Jackson & Perkins 2001) and the normal variation in biological tissues may contribute to the standard deviation. It has been shown that the tissue can be frozen during storage without affecting the mucus layer (Bredenberg & Nystrom 2003) but freezing and excessive handling increase the risk of changing the properties

of the mucus layer. For dosage forms that adhere as a result of hydration of the dosage form it has been found that the mucous tissue can be replaced with other surfaces without changing the relative adhesive strength (Mortazavi & Smart 1995; Jacques & Buri 1997). The alternative to excised tissues is to use a mucus preparation. Purified mucins, which are commercially available, may reduce the variations in results. However, the purification procedure most likely degrades the mucin molecules and alters their properties (Madsen et al 1996; Kocevar-Nared et al 1997), which may lead to results that do not reflect the interaction and adhesion that occurs in the in-vivo situation.

*In-vivo methods*

In-vivo methods for studying bioadhesion are relatively scarce. Some methods assess the residence time at the application site using gamma scintigraphy (Davis 1986; Harris et al 1990; Richardson et al 1996; Brown et al 1997; Soane et al 1999, 2001; Chatterton et al 2004) or dyes (Nakamura et al 1996), while others involve measurement of the transit time using radioisotopes (Ch'ng et al 1985; Riley et al 2001). The successful use of tracers added to the formulation relies upon the properties of the vehicle remaining unchanged and, therefore, behaving in a manner that is identical to that in the absence of the tracer so that the results obtained are a genuine reflection of the residence time of the dosage form. Possible reasons for the small usage of in-vivo methods are that they cannot discriminate between mucoadhesion and other factors affecting the residence time, they are expensive and they are often accompanied by large standard deviations.

*Detachment force*

The detachment force method is based on measurement of the tensile, peel or shear stress when detaching a formulation from tissue (Figure 3). Peel adhesion tests are mainly used for buccal (Guo & Cremer 1999) and transdermal patches (Horstmann et al 1999). Shear force measurements have been widely employed, but by far the most common test is the tensile adhesion test.

In principle, when conducting tensile adhesion tests, the formulation is brought into contact with a biological substrate and the force or the work that is required to break the adhesive bond is measured. The instruments used for tensile adhesion tests are usually modified balances or tensile testers. The biological substrate could be excised tissue or a mucus preparation. There are several methods that have been developed for different kinds of formulations: dry tablets (Ponchel et al 1987; Lejoyeux et al 1989; Jacques & Buri 1992; Wong et al 1999), disks (Robert et al 1988; Smart 1991; Chen & Hwang 1992), powder/granules/particles (Ch'ng et al 1985; Park & Robinson 1985; Bredenberg & Nystrom 2003) and semi-solid vehicles (Caramella et al 1994; Jones et al 1997; Tamburic & Craig 1997; Hagerstrom & Edsman 2001). The tensile adhesion test reproduces processes similar to those that might occur in-vivo, although in reality, tensile detachments are probably relatively rare because shear forces or a combination of forces are most likely to act on the vehicle. Nevertheless, these measurements give information about the strength of the combined mucoadhesive complex, even though the type of detachment is not exactly the same as in-vivo. As in the in-vivo situation, these tests do not always give a measure of the interaction between the formulation and the mucus layer, but also of factors related to the water transport between the dosage form and the mucosa. The force measured will reflect the weakest link in the set up, which may be the formulation itself, the hydrating dosage form, the mucus layer or the interfacial region. The different layers will contribute to a different extent depending on the formulation, and it has been shown that the detachment force depends on the source of the mucosa (Jackson & Perkins 2001).

More information about the interaction can be accumulated using this method, because it not only provides a value for the work of adhesion or the peak force, but also for the different deformation parameters (Figure 4) (Chickering &

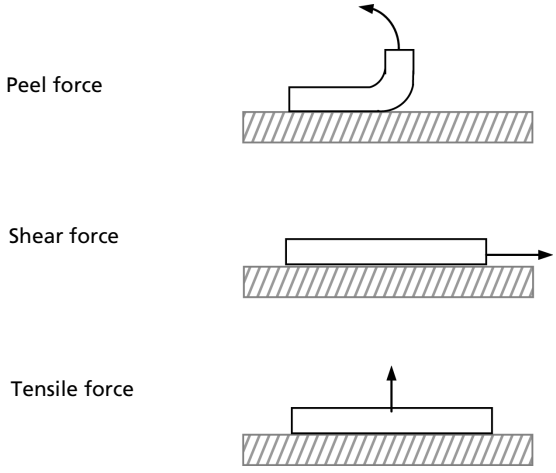


**Figure 3** Representation of the peel, shear and tensile forces that can be determined when measuring the adhesive bond strengths.

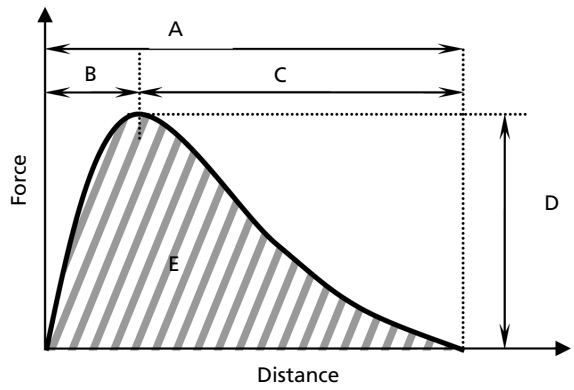


**Figure 4** Force distance curve, defining deformation to failure (A), deformation to peak (B), deformation peak to failure (C), peak force (D) and tensile work (E).

Mathiowitz 1995; Hagerstrom & Edsman 2001), which can supply information of value for the interpretation of data. It has been suggested that the tensile deformations measured depend on the mechanical properties of the tissue, the mucoadhesive material and the adhesive bond.

*Rheological method*

For polymer solutions and gels, Hassan & Gallo (1990) proposed a simple rheological method for measuring mucoadhesion. In this method, the interpenetration layer is simulated by mixing the polymer solution with a mucin solution. A rheological parameter, such as the viscosity or the elasticity, is measured for the mixture and this value is compared with the rheological properties of the polymer and the mucin separately (Figure 5). If the value for the mixture is larger than the sum of the values for the polymer and the mucin, it is assumed that an interaction based on entanglements, conformational changes and chemical interactions has occurred to produce a change in the rheological behaviour.

The rheological method was first described for viscosity measurements, but has since been adopted for other rheological techniques and, in particular, it is often employed for viscoelastic parameters.

The rheological method has been extensively used for mucoadhesion studies, but it is not always easy to interpret the results. A wide variation in results is found in the literature, and the results depend on the concentration of the mucin and the type of mucin used (Rossi et al 1995; Madsen et al 1996; Kocevar-Nared et al 1997; Hagerstrom et al 2000), as well as the concentration of the polymer (Mortazavi & Smart 1994a; Madsen et al 1998; Hagerstrom et al 2000) and experimental parameters (Hagerstrom et al 2000; Hagerstrom & Edsman 2003). Another issue is that a negative interaction parameter does not always imply the absence of an interaction, and a strong interaction on the molecular scale does not always produce a strengthening of the macroscopic rheological behaviour (Williams & Phillips 1995; Nishinari et al 1996; Rodriguez et al 2001).

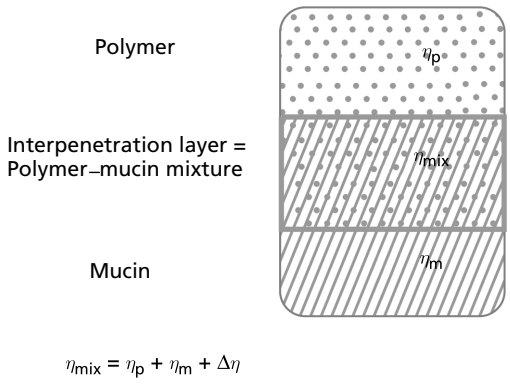


**Figure 5** The basis of the rheological method. $\eta_p$, viscosity of polymer; $\eta_{mix}$, viscosity of polymer–mucin mixture; $\eta_m$ viscosity of mucin; $\Delta\eta$, the interaction parameter.

A modification of the method has been made by Rossi et al (1999b), whereby data from creep measurements were analysed using mechanical models in an attempt to obtain more information about the types and strength of interactions between the polymer and mucin. A description of the models and calculations used for the treatment of the rheological data was published by Ironi & Tentoni (2003).

A rheological technique has also been used for powder formulations to assess interactions with mucins (Ceulemans & Ludwig 2002) and was subsequently used to study the mucoadhesive properties of some nasal insulin powder formulations based on starch and carbopol (Callens et al 2003b). The results did not provide evidence of any rheological interactions between the formulations and mucin, with the exception of a formulation with one of the lowest bioavailabilities, where entanglements with mucin were observed. The in-vivo bioavailability of insulin seemed to correlate better with the elastic and viscous properties of the formulation.

*Flow retention techniques*

In several methods, the formulation is brought into contact with an excised tissue and then exposed to a flow of either air or liquid, whereupon the retention of the formulation is measured. The detection method used depends on the type of formulation and can consist of such measurements as the time taken for the formulation to pass over the tissue, the amount retained after a certain time (Ranga Rao & Buri 1989) or the distance travelled by the particle or formulation during a certain amount of time (Mikos & Peppas 1990). This method is especially useful for particles (Teng & Ho 1987; Ranga Rao & Buri 1989), liquid formulations (Dobrozsi et al 1999; Batchelor et al 2002) and gels (NguyenXuan et al 1996; Le Ray et al 1999).

*Measurements of surface energies*

Measurements of surface tension and spreading coefficients are based on the wetting theory of mucoadhesion (Table 1). Attempts have been made to correlate the contact angles to mucoadhesion (Lehr et al 1992a). Later, Lehr et al (1993) developed a combined spreading coefficient, defined by the geometric mean of the polymer spreading coefficient and the Griffith fracture energy. It has been shown that the combined spreading coefficient, calculated from contact angle measurements, correlates to the force of detachment (Lehr et al 1993; Shojaei & Li 1997). The interfacial energies that are involved in the mucoadhesion process are illustrated in Figure 6. For a given mucosal tissue and surrounding body liquid, the free surface energy of the mucoadhesive dosage form should be intermediate between the free surface energy of the surrounding liquid and that of the mucosal surface. If the free energy obtained for the dosage form is too high, the formation of a dosage–liquid interface will be favoured, preventing the intended adhesion to the mucosal surface. Dosage forms with free surface energies that are lower than that of the mucosal tissue surface will not spread on the tissue, and hence will not adhere to the mucosal surface. If the free surface energies of the dosage

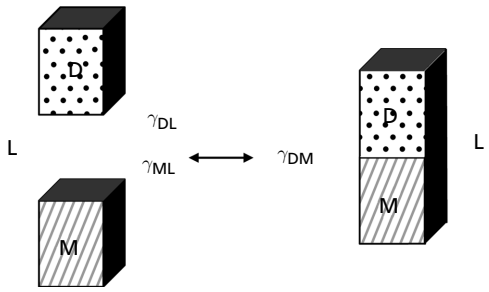


**Figure 6** The interfacial energies, given by $\gamma$, involved in the formation of a mucoadhesive bond. D, dosage form; M, mucosa; L, surrounding liquid.

form and the mucosa are very similar, the mucoadhesion will be very weak.

*Miscellaneous methods*

Different surface analysis methods have been used to study mucoadhesion. For example, ATR-FTIR has been used in several studies to investigate the interfacial layer between the dosage form and the mucus. Jabbari et al (1993) studied the interpenetration between a film of PAA and mucin. A film of the polymer was placed on the ATR crystal and a buffered mucin solution was then put onto the polymer film. The absorption bands in the ATR spectrum were used to monitor the concentration of mucin in the interpenetration layer. The ATR-FTIR technique has also been used to monitor the diffusion of water and mucin from a mucin solution into a polymer film (Saiano et al 2002). Changes in the spectra were observed as a result of hydrogen bonding between the mucin and the polymers. The areas corresponding to water, polymer and mucin were integrated at different times and revealed that the water and mucin wet and penetrate the polymer matrix simultaneously. ATR-FTIR studies have also been used to show how the monomer composition in copolymers influences the formation of intermolecular and intramolecular hydrogen bonds (Shojaei & Li 1997). When poly (ethylene glycol) (PEG) was introduced into PAA the formation of hydrogen bonds was enhanced.

In addition to the techniques discussed above, different microscopic methods have been used to visualise the structure and organisation of mucin alone and in mixtures with potential mucoadhesives. For example both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have been used to investigate the interaction of mucin with chitosan solution (Fiebrig et al 1995) and chitosan microspheres (Genta et al 1998). Furthermore, atomic force microscopy (AFM) has been used to study aggregates of polymers and mucin (Deacon et al 2000) and polymer adsorption to cell surfaces (Patel et al 2000). NMR microscopy has been used to study the diffusion of water in the bioadhesive bond between alginate and a mucin solution (Marshall et al 2001).

The interaction between mucin and cellulose derivatives has been measured using ellipsometry (Malmsten et al 1994) by measuring the thickness of the polymer layer adsorbed onto a mucin coated surface.

Fluorescence techniques have been used to study mucoadhesion. Park & Robinson (1984) used pyrene to label the lipid bilayer of cells to study the adhesion of polymers to cells and Quaqish & Amiji (1999) used fluorescein isothiocyanate (FITC)-labelled chitosans to study the interaction with mucin.

Finally, another approach to the investigation of the mucoadhesive interface that has been attempted recently is to study the compatibility between the formulation and the mucosa by measuring the ease with which ionic species are transported across the interface using dielectric spectroscopy (Hagerstrom et al 2003).

## Factors influencing mucoadhesion

Several properties of the polymer in the dosage form are known to affect the formation of intimate contact and participation in interactions. Much of the classification of the important factors was published in the early days of mucoadhesion research or stems from adhesion science and appears in many reviews of bioadhesion (Junginger 1991; Jimenezcastellanos et al 1993; Gandhi & Robinson 1994; Ahuja et al 1997; Lee et al 2000; Vasir et al 2003) (Table 2). The factors are in many ways logical when thinking of the mechanisms suggested to be involved in the bioadhesion process. There are, however, also factors that are related to the route by which the drug is administered, and the properties and turnover rate of the mucus.

As the research on mucoadhesion has grown and been compiled, it has become increasingly apparent that mucoadhesion is a complex phenomenon to investigate because of the dependence of the results on the experimental setup, the type of formulation (e.g. if it is dry or hydrated), the swelling characteristics and how the dosage form is applied. There are also factors that are related to the method used for the mucoadhesion measurements. If the mucoadhesion method is a measurement of the force or work required to detach the formulation from the tissue, the measured work or force will be dependent on where the failure occurred in the mucoadhesive complex and will reflect the cohesive nature of the dosage form, the hydrating layer, the interfacial region or the strengthened mucus layer. In contrast, other methods, such as the rheological method, which simulates the interpenetration layer by mixing the mucin and the polymer, will be dependent on factors affecting the rheological properties of the mixture.

*Molecular weight*

The molecular weight dependence of mucoadhesion is not straightforward because the results from different measurements vary with the type of polymer and the dosage form used in the study as well as the method of measurement.

For systems using solid or dry dosage forms, the molecular weight dependence varies with the type of polymer. For example, the detachment force for PAA was maximal at a molecular weight of 1 000 000 (Tobyn et al 1996) while in the same study, conducted on different viscosity grades of carboxymethylcellulose (CMC), the detachment force

**Table 2** Factors affecting mucoadhesive properties of polymers, as traditionally reported

| Property | Effect on mucoadhesion | Classic references |
|---|---|---|
| Molecular weight | There is an optimum molecular weight for mucoadhesion at which the chains are small enough to allow an easy interpenetration, but also large enough for entanglements to occur. This optimum molecular weight is different for different polymers as it depends on the flexibility and the conformation of the polymer chain. | Huntsberger 1967; Chen & Cyr 1970; Smart et al 1984; Duchene et al 1988 |
| Chain flexibility | The chain flexibility will be affected by the type of polymer, the concentration and the cross-linking density. A high flexibility will increase the possibilities of interpenetration. | |
| Concentration | There is an optimum polymer concentration for hydrated formulations at which the adhesive properties are the best. At very high concentrations, the molecules tend to be coiled, reducing the flexibility and therefore also decreasing the possibility of interpenetration and the formation of entanglements. At too low concentrations there are not enough chains available for interaction. For solid formulations it has been shown that the adhesion increases with increasing concentration of the polymer. | Gurny et al 1984; Duchene et al 1988 |
| Cross-linking density | A high cross-linking density reduces the flexibility of the polymer chains and hence also the capacity for interpenetration and the formation of entanglements. A high degree of cross-linking will give less adhesion to the mucus. | |
| Presence of chemical groups, charge and ionization | The presence of chemical groups that may aid in the formation of interactions between the polymer and the mucus will be favourable for mucoadhesion. Sufficient quantities of chemical groups that form hydrogen bonds with the mucus gel, such as hydroxyl, amine, sulfate and carboxyl groups, increase the adhesion. The charge density has also proved to be important. In addition, it has been suggested that, when considering toxicity as well as bioadhesion, polyanions are preferred before polycations and carboxyl containing polymers are better than sulfated ones. Since the pH (and the relationship between the pH and the $pK_a$) affects both the charge density of the mucin molecule and the polymer, the mucoadhesion will be affected. | Park & Robinson 1984; Ch'ng et al 1985; Park & Robinson 1985 |
| Swelling | The swelling of the polymer depends on the concentration of polymer, ionic strength and the presence of water. Consequently, the mucoadhesion will have a maximum at optimal water content and overhydration reduces the adhesion. | Chen & Cyr 1970; Leung & Robinson 1990 |

increased with increasing molecular weight for all molecular weights in the study. These results were explained by two factors that influence the properties of the tablets. Firstly, there is better cohesion (measured by self-adhesion) for tablets made from the higher-molecular-weight polymer. Secondly, an opposing effect arises because the aqueous dispersibility decreases with increasing molecular weight, which leads to fewer solubilised carboxylic acids groups being available for hydrogen bonding for the higher-molecular-weight than the lower-molecular-weight polymers. In addition, the ability to take up water from the tissue is lower for the higher molecular weights. In contrast to the optimum found for PAA, the absence of an optimum in measurements on CMC was explained by the fact that CMC has a good solubility independent of its molecular weight, leaving only the cohesion factor to determine the molecular weight dependence, and the cohesion factor favours the high-molecular-weight material.

Rossi et al (1996), however, found the opposite molecular weight dependence for CMC, with greater mucoadhesion being apparent for lower molecular weights, for isoviscous solutions of CMC, using both the rheological method and a detachment force method using polymer- and mucin-soaked filter paper discs.

Di Colo et al (2001a) studied the molecular weight dependence for poly(ethylene oxide) (PEO) gel-forming inserts, and discovered that the work of detachment was inversely proportional to the molecular weight in the molecular weight range 200–2000 kDa, but that the bioavailability of ofloxacin in the eye increased as the molecular weight of the PEO in the insert increased (200–900 kDa). The suggested explanation put forward for this inverse relationship between bioavailability and mucoadhesion was that the viscosity of the tear fluid increases more with a higher-molecular-weight PEO, thereby increasing the contact time of the drug in the eye.

Leitner et al (2003a) showed that there is a positive correlation between the force of detachment and molecular weight for tablets made from PAA-cysteine conjugates – the mucoadhesion increased with the molecular weight of the PAA-cysteine conjugate (from 2000 to 450 000 Da).

Another factor that varies with the size of the molecule is the number of molecules involved in the interaction. By using FITC-labelled chitosans of different molecular weight (70 000–2 000 000 Da), it has been shown that high-molecular-weight chitosan offers multiple sites for mucin attachment (Qaqish & Amiji 1999), while low-molecular-weight polymers associate univalently.

*Concentration*

The effect of polymer concentration on the mucoadhesive properties is more complex than suggested in Table 2 – for hydrated and liquid dosage forms, the cohesive nature of the liquid or semisolid vehicle is of importance. Increasing the concentration will increase the cohesion of the formulation, and hence, the force or work of detachment for weak dosage forms. Above the isotonic concentration, an increase in the concentration will also increase the water transport from the mucosa to the dosage form and hence strengthen the mucus layer. In addition, the concentration will affect the possibilities for interpenetration and the strength of interactions.

In a study of pharmaceutical gels, Jones et al (1997) showed that an increase in the mucoadhesive properties of hydroxyethylcellulose (HEC) and CMC occurred as a result of increasing concentration. However, as dry compacts of mucin were used for these measurements, the strength of the mucoadhesive bond increased up to a maximum concentration. Beyond that concentration, the amount of free water in the gels available to produce swelling of the mucin decreased, reducing the strength of the mucin–gel adhesive bond.

In another study, the work and force of adhesion increased with increasing concentration, especially for high-molecular-weight polymers or covalently cross-linked polymers (Hagerstrom & Edsman 2001). A multivariate analysis of the work of adhesion and the tensile force revealed that the rheological properties of the formulations are important for both the mucoadhesion and the cohesion of the formulations (Hagerstrom et al 2004); the rheological properties are largely determined by the concentration and the molecular weight of the polymers.

*Cross-linking density*

The cross-linking density has an effect on mucoadhesion that varies with the dosage form used and whether this is dry or fully hydrated. Furthermore, for fully hydrated vehicles formed from polymers with ionisable groups, the result can depend on the pH used in the study. The swelling ability of these hydrogels depends on the cross-linking density and on the presence of ionised or non-ionised groups in the hydrogel. The swelling ability affects both the strength of the hydrating layer of the dosage form and the dehydration of the mucus, as well as the mobility of the polymer chains.

PAAs have been used in several studies. Park & Robinson (1987) studied the force of detachment of hydrated PAA microparticles at gastric pH and found an inverse relationship between the detachment force and the cross-linking density (between 0.1 and 2% cross-linking density). Warren & Kellaway (1998), on the other hand, found that the force of detachment increased with an increasing cross-link density when using fully hydrated and neutralized PAA gels. For compressed tablets of PAA, Tobyn et al (1996) showed that the detachment force for cross-linked PAA was significantly greater than for the non-cross-linked polymer.

In contrast, there are several studies that have revealed the cross-linking density to have no effect on mucoadhesion. Achar & Peppas (1994) did not find that the cross-linking density for microparticles made from copolymers of methacrylic acid and methylmethacrylate had any influence on the mucoadhesive force using a flow channel method. Nor did DeAscentiis et al (1995) find any dependence of the cross-linking density (0–0.05 mol cross-link/mol monomer) for poly(hydroxyethyl methacrylate) (PHEMA) microspheres on the adhesion to rat intestinal mucosa.

If the cross-linking reaction changes the chemical structure of the polymer, the mucoadhesive properties will be affected. By using microscopy, Genta et al (1998) showed that mucin had a high affinity for non-cross-linked microparticles of chitosan compared with glutaraldehyde cross-linked chitosan microspheres. Since glutaraldehyde reacts with the amino groups in the chitosan molecule, there are fewer possibilities for interaction between the amino groups and the mucin.

Attempts have been made to increase the mucoadhesion by adding linear polymers to cross-linked gels. Sahlin & Peppas (1997), for example, added linear PEG to cross-linked PAA and thereby increased the adhesion between two hydrogels by two orders of magnitude despite the fact that this had a negative impact on the wetting characteristics of the hydrogels. With near-field FTIR microscopy, they showed that PEG diffused across the boundary between the hydrogels.

*Chemical structure*

The chemical structure of the mucoadhesive will influence several properties that are important for mucoadhesion. Firstly, it will affect which type of interactions can occur between the mucus layer or mucin molecules and the polymer. Secondly, the hydrophilicity of the polymer will affect the ability to take up water. The swelling of a dry, or not fully hydrated, polymer will, in turn, give rise to both strengthening of the mucus layer by dehydration and weakening of the dosage form by water uptake. For polymers containing ionisable groups, pH dependence of the mucoadhesion can be expected.

Hydrogen bonds are an important feature of the interaction between mucin and PAA at low pH (Tobyn et al 1992) and can explain the observed pH dependence of the mucoadhesion of PAA (Park & Robinson 1985). Furthermore, it has been shown that addition of hydrogen-bond-breaking agents to carbopol and PEO discs resulted in a reduction of the mucoadhesive strength, providing indirect evidence of the importance of hydrogen bonds (Mortazavi 1995). The presence of hydrogen bonds in the interpenetration layer has been shown using ATR-FTIR (Saiano et al 2002).

Electrostatic interactions are another kind of interaction that plays an important role in the mucoadhesion process. In a study of the mucoadhesion of cross-linked microparticles made from hydrophilic polymers, it was found that the adhesion increased with an increase in the number of ionic components in the copolymer (DeAscentiis et al 1995). Several studies have revealed the existence of a charge-related effect on the polymer. For example, Bogataj et al (2003) found a correlation between the zeta potential and the force of detachment

from pig vesical mucosa, which is highly negatively charged. A stronger interaction was seen for positively charged polymers. In a study by Jackson & Perkins (2001) of ion-exchange resins of different charge, positively charged resins showed a higher force of detachment from pig or human gastric mucosa than negatively charged resins. The effect of charge of the polymer has also been shown using polymeric films. Films made from the positively charged chitosan had a significantly higher force of detachment than the two negatively charged polymers, polycarbophil and CMC. Deacon et al (2000) used AFM to study the interaction between chitosan and pig gastric mucin at a variety of ionic strengths, showing that electrostatic interactions are important for promoting mucoadhesive interactions at physiological ionic strength.

It has also been shown that positively charged liposomes were more strongly adhesive than negatively charged ones (Takeuchi et al 2003). Furthermore, for polymer-coated liposomes, greater mucoadhesion was noticed when the liposomes were coated with the cationic chitosan instead of the negatively charged carbopol. The adhesion of carbopol-coated liposomes decreased when increasing the pH from 5 to 7.4 because of electrostatic repulsion between the liposomes and the mucus layer.

The presence of cations can influence the mucoadhesion of PAA derivatives (Lejoyeux et al 1989; Kerec et al 2002). On sublingual mucosa, the detachment force and the work of adhesion decreased with an increasing calcium chloride concentration in the test medium, but on vaginal mucosa calcium had no significant influence on the adhesion (Lejoyeux et al 1989). Kerec et al (2002) studied the effect of calcium ions on the force required to detach polycarbophil microspheres using mucosa from urinary bladders. While sodium ions had no influence, the presence of calcium ions lowered the detachment force. This effect was probably caused by chelation and cross-linking of the polymers, reducing the flexibility of the polymer as well as the interaction of the carboxylic groups of the polymer with the mucin.

The interactions observed between the mucoadhesive polymer and mucin could often be attributed to a combination of several interactions. In a study of the interaction between chitosan and mucin at different values of pH and ionic strength (Qaqish & Amiji 1999), it was shown that there might be multiple modes of interaction involving hydrogen bonding and hydrophobic interactions, in addition to the electrostatic interactions.

Another possible type of interaction is the formation of covalent bonds within the polymer itself and between the mucoadhesive polymer and the mucin, which has been investigated using thiolated polymers (Bernkop-Schnurch & Steininger 2000; Leitner et al 2003a, 2003b).

*Particle size*

The size of mucoadhesive particles may influence their mucoadhesion. It has therefore been suggested that comparisons of potentially mucoadhesive systems should be done at constant particle size. Mikos et al (1991) calculated the various forces that act on a particle when interacting with the mucosa and concluded that the mucoadhesive force exerted on a particle was dependent on the viscoelastic properties of the mucosa and increased with particle diameter. Achar & Peppas (1994) have also demonstrated that the results from flow retention studies will depend on the size of the microspheres (400–1000 $\mu$m) because of the difference in the surface area in contact with the mucosa. From adsorption studies, Ponchel et al (1997) have suggested a particle adsorption model where particles of less than 1 $\mu$m will penetrate into the mucus layer, while larger particles exhibit an adsorption pattern that is indicative of a monolayer of particles on the mucus surface. There are also studies where no influence of particle size ($<40$, 40–90 and 90–125 $\mu$m) was found (Jackson & Perkins 2001).

## Routes of administration

In this section the most common mucosal administration routes will be described together with examples of in-vivo studies of mucoadhesive dosage forms. The mucosal routes are traditionally used for local treatment, but are also used and explored for systemic delivery of drugs. An advantage of systemic administration via one of these routes, compared with oral administration, is the avoidance of first-pass metabolism.

In the examples given, the reported increase in residence times and bioavailabilities may very well be caused by factors other than mucoadhesion. For example, for liquid vehicles, an increase in viscosity will prolong the residence time for most administration routes. Depending on the chosen reference, the improvement may be the result of a combination of mucoadhesion and the increase in viscosity. For solid dosage forms, the bioavailability will be dependent on both the residence time and the release rate of the drug. The reported bioavailability will, therefore, be affected by several factors other than mucoadhesion. The polymers of the mucoadhesive dosage forms may also have some effect besides that of creating intimate and prolonged contact at the site of administration, for example, chitosan has been shown to enhance permeability (Artursson et al 1994; Schipper et al 1996, 1997; Dodane et al 1999; Senel et al 2000; Tengamnuay et al 2000; Sinswat & Tengamnuay 2003; Di Colo et al 2004; Sandri et al 2004).

It is difficult to design a study of the mechanisms involved and not many studies have evaluated which factors contribute to the observed improvement. In this section, therefore, the results from the different studies will be reviewed as examples of what can be achieved, but without discussing the possible mechanisms behind the results.

*The buccal route*

The oral cavity is used both for systemic delivery and local treatment. Systemic delivery of drugs is either sublingual, through the mucosal membranes lining the floor of the mouth, or buccal, through the mucosal membranes lining the cheeks. The total surface area of the oral cavity is approximately 100 $cm^2$, of which the buccal mucosa represents approximately one-third. The epithelium of the oral mucosa consists of a stratified squamous epithelium (Figure 7), the

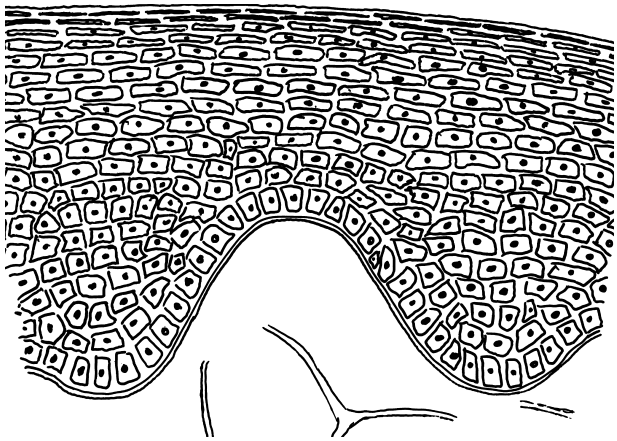

**Figure 7** Schematic drawing of the buccal mucosa.

thickness of which varies depending on the site. In the buccal region the epithelium is around 40–50 cells thick, whereas it is somewhat thinner in the sublingual area. In areas subject to mechanical stress (e.g. the gingival and the hard palate) the mucosa is keratinised in contrast to the mucosa of the sublingual and buccal regions. The mucus in the oral cavity is secreted by salivary glands as a component of the saliva and is adsorbed to the surface of the oral mucosa, forming a 0.1- to 0.7-mm thick layer. Sublingual mucosa is more permeable than buccal mucosa, but because of the large production of saliva sublingual administration is considered to be difficult for formulations intended to act over a long period of time. It is therefore mainly used for treatments requiring a rapid onset and of short duration. The buccal route, on the other hand, has been studied for sustained delivery of drugs using adhesive dosage forms, for example, in gels, ointments, patches and tablets. Among the mucoadhesive dosage forms tablets and patches are the most studied.

Tablets used for local delivery of drugs to the oral cavity are often based on linear or cross-linked polymers, which release the drug as a result of hydration and erosion. The tablets usually consist of one layer with the same composition throughout the whole tablet and have a residence time of several hours in the oral cavity. When using erodible discs made from CMC and hydroxypropylmethylcellulose (HPMC) (Ali et al 2002), developed for overnight treatment of oro-dental infections, the salivary concentration of cetylpyridinium chloride was maintained above the minimum inhibitory concentration (MIC) for 8 h in healthy subjects. Clotrimazole gave effective salivary drug levels over a period of 6 h using buccal tablets made of PAA and HPMC (Khanna et al 1997). Other examples of in-vivo studies of mucoadhesives for local treatment are the delivery of miconazole from tablets based on PAA in healthy subjects (Bouckaert et al 1993), lactoferrin in buccoadhesive tablets based on sodium alginate (Kuipers et al 2002) and chlorhexidine-containing PAA/HEC tablets (Irwin et al 2003).

Mucoadhesive buccal tablets have been used as an alternative to oral delivery for systemic delivery of drugs undergoing extensive first-pass metabolism. For example, the bioavailability of testosterone in dogs increased to 14% when delivered from mucoadhesive tablets compared with 1% if given orally (Voorspoels et al 1996). Bilayer tablets with a rapidly dissolving layer on a mucoadhesive formulation have been used to achieve biphasic release of nicotine. The mucoadhesive layer contained carbopol and hydroxypropylcellulose (HPC) and released nicotine for 4 h in healthy subjects (Park & Munday 2002). Buccoadhesive nifedipine tablets based on CMC and carbopol (Varshosaz & Dehghan 2002) adhered to the upper gums of humans for over 8 h. Other examples are the delivery of morphine sulfate, from tablets based on HPMC and PAA, in healthy subjects (Anlar et al 1994) and the delivery of thiocolchicoside from mucoadhesive tablet based on CMC and gelatin (Artusi et al 2003). Although the buccal mucosa is considered to be more resistant to damage than other mucosal membranes, serious irritation and ulceration can occur as a result of local toxicity, as in the case of buccal delivery of propranolol hydrochloride (Taylan et al 1996). One disadvantage of the single-layer tablet for systemic delivery is that the drug will also be released into the saliva and swallowed. To improve the bioavailability, it is possible to direct the release to the mucosa by layering the tablet with an impermeable coating on the side facing the salivary flow, thereby reducing the amount of drug being dissolved in the mouth. An example of a multilayered mucoadhesive tablet is the one used for sustained delivery of chlorpheniramine maleate in rabbits (Alur et al 1999a), which was coated on all sides but one to direct the delivery of the drug to the mucosa.

Adhesive films and laminated patches are considered more user friendly than buccal tablets since they are smaller and more flexible than the tablets. As for tablets, an impermeable backing layer will increase the adhesion time and bioavailability by retarding the diffusion of saliva into the formulation and the drug release into the mouth. In a study of buccal delivery of testosterone in rabbits, a bilayer composite patch resulted in a bioavailability of 50% (Jay et al 2002). Patches have also been used for local delivery of miconazole, which gave effective salivary levels over at least 6 h in healthy subjects (Nafee et al 2003).

The buccal route has also been investigated for systemic delivery of peptides and proteins. Salmon calcitonin was delivered from buccal tablets in rabbits using a polysaccharide from *Hakea gibbosa* (Alur et al 1999b). To direct the delivery of the drug to the mucosa, the tablets were coated on all sides but the one that attaches to the mucosa. It was found that biologically active salmon calcitonin was delivered across rabbit buccal mucosa with an apparent bioavailability of 37%. Calcitonin was also delivered from a thin film composite in rabbits with a bioavailability of 44% (Cui & Mumper 2002a). Other examples of drugs tested are oxytocin (Li et al 1997a) and thyrotropin-releasing-hormone (TRH) (Li et al 1997b). The buccal route has also been explored for mucosal immunisation by Cui & Mumper (2002b). Bilayer films were applied to the buccal pouch of rabbits once a week for three weeks and resulted in comparable or better

serum total IgG titres than were achieved using subcutaneous injection.

Mucoadhesive gels have also been evaluated for delivery in the oral cavity. For the delivery of tetracycline hydrochloride to the periodontal pocket, a combination of HEC, polycarbophil and poly(vinyl pyrrolidone) (PVP) significantly reduced the amount of potentially pathogenic bacteria at the periodontal site (Jones et al 2000).

*The nasal route*

The basic function of the nose, in addition to functioning as a sensory organ, is the pretreatment of inspired air. The air is heated and humidified, and its passage through the nose will help clear particles and bacteria from the air before it reaches the lung. The outermost part of the nose is the nasal vestibule. The nasal cavity, which has a length of 60 mm and a volume of approximately 20 mL, is divided vertically by the nasal septum for most of its length. Each of the cavity walls contains three folds, known as the nasal turbinates, which give the nasal cavity a relatively large surface area of approximately 160 $cm^2$. The large surface area, in combination with a well-vascularised tissue, are factors that make the nasal cavity interesting, not only for local treatment, but also for drug absorption to the systemic circulation. Most of the nasal cavity is lined with mucous membrane containing columnar cells, goblet cells and basal cells (Figure 8). In the anterior part of the nasal cavity, the columnar cells are non-ciliated, whereas in the remainder of the nasal cavity they are covered with cilia. Each cell has around 300 cilia, 5–10 $\mu$m long, beating in regular waves with a frequency of 10 Hz. The cilia are responsible for mucociliary clearance from the nasal cavity to the nasopharynx, for further transport to the gastrointestinal tract. The mucociliary clearance is a part of the defensive functions of the nose, transporting particles, bacteria and dissolved substances, to prevent them from reaching the respiratory tract. Normally the mucus layer is 5–20 $\mu$m thick and is divided into two layers, where the outer layer has a high viscosity and a gel-like character, while the layer closest to the cells has a lower viscosity enabling the cilia to move. The turnover time for mucus is usually given as 10–15 min, but it is affected by both environmental conditions and diseases. The rapid mucociliary clearance is among the main drawbacks of nasal drug delivery, together with the local toxicity, the presence of proteolytical enzymes and variations caused by pathological conditions. Among the advantages, on the other hand, are the relatively rapid uptake and the avoidance of the first pass metabolism. This route is, however, less suited for sustained delivery of drugs. The most common dosage forms are solutions, gels and particles. A few studies, though, have shown that there may be toxicological issues with some of the dosage forms (Ugwoke et al 2000b, c; Callens et al 2001).

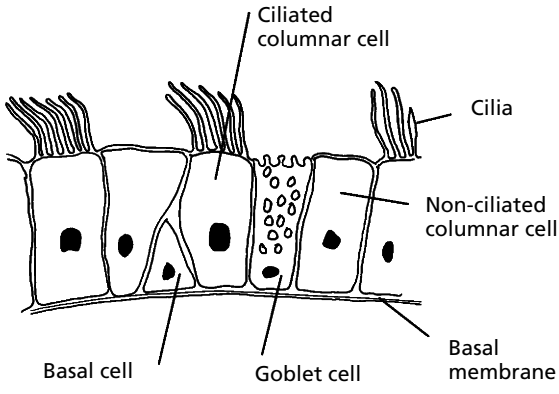


**Figure 8** Schematic drawing of the nasal epithelium.

From a study of the mucociliary clearance of polymer gels in rats, investigated by fluorescently labelled microspheres (Zhou & Donovan 1996), it was concluded that formulations that are either very viscous or very fluid undergo rapid clearance during the first 60 min. Even with this rapid initial clearance, formulations with a strong bioadhesive capacity can significantly limit the total clearance from the nasal cavity. In a gamma scintigraphy study in man (Soane et al 1999), a 1% chitosan solution had a clearance half-life of 41 min, which should be compared with the reference solution, which had a half-life of 21 min. In a study of the clinical efficacy of decongestant formulations, Tzachev et al (2002) compared a standard solution with a mucoadhesive solution based on chitosan, and found that the mucoadhesive solution was both more effective and longer lasting than the reference.

Liposomes have also been used for nasal delivery of active substances. For example, a prolonged plasma concentration of nifedipine (Vyas et al 1995) and a prolonged antihistaminic effect of diphenhydramine hydrochloride (Iwanaga et al 2000) were obtained as a result of the increased drug retention in the nasal cavity.

Microparticles are the most studied mucoadhesive dosage form for nasal delivery, with retention times of several hours. In a gamma scintigraphy study (Vidgren et al 1991), the clearance of disodium cromoglicate particles and disodium-cromoglicate-loaded PAA microparticles in man was investigated. After 30 min, 27% of the plain drug particles and 50% of the PAA microparticles were retained. Powders of water-soluble polymers (HPC, xanthan gum, tamarind gum and polyvinyl alcohol (PVA)) were evaluated in-vivo in rabbits (Nakamura et al 1996) using a dye (Brilliant blue). As a solution, the dye disappeared completely within 2 h, while the polymer powder was retained for 4–6 h, with the exception of PVA, which was completely lost 4 h after the start of the measurements. Ugwoke et al (1999a, b, 2000a, d) have studied microspheres of lactose (as the control), cross-linked PAA derivatives and CMC as vehicles for apomorphine in rabbits. The intranasal clearance was 58% after 3 h for the formulation with lactose, 12% for carbopol 971 and 27% for CMC (Ugwoke et al 2000d), but the empty vehicles had a faster clearance, which was probably caused by the cilio-inhibitory action of the drug. Both CMC and carbopol sustained the plasma concentrations and increased the bioavailability of apomorphine to levels comparable with those achieved after subcutaneous injection (Ugwoke et al 1999a, 1999b, 2000a). Starch and chitosan microspheres

had a clearance half-life of 68 and 84 min, respectively, in man, when studied by gamma scintigraphy (Soane et al 1999). In a later study by the same group (Soane et al 2001), of the clearance of microspheres in sheep, a clearance half-life of 115 min was found for the chitosan microspheres and 43 min for the chitosan solution, which correlated well to the human study. Gentamicin delivered in biodegradable microspheres made from hyaluronate or chitosan hydroglutamate, or microspheres made from both polymers, resulted in increased bioavailability in rabbits (23%, 31% and 43%, respectively), compared with a gentamicin solution (1.1%) and gentamicin dry powder (2.1%) (Lim et al 2002).

Microspheres have also been investigated as vehicles for nasal delivery of larger molecules, such as peptides and proteins. An increased bioavailability of FITC-dextran (MW 4300) was observed in rats when microspheres made from carbopol were used as a vehicle in comparison with reference lactose microspheres (Abd El-Shafy et al 2000). A significantly greater hypocalcaemic effect was observed after administration of salmon calcitonin in gelatin microspheres in comparison with salmon calcitonin in buffer (Morimoto et al 2001). The bioavailability of salmon calcitonin was greater when using positively charged spheres (made from basic gelatin) than when using negatively charged spheres (made from acidic gelatin) of the same size. Bioadhesive starch microparticles have been found to synergistically increase the effect of absorption enhancers on transport of insulin across the nasal membrane in sheep (Illum et al 2001). Long-term use of mucoadhesives, however, may reduce bioavailability. For example, after 8 days of nasal delivery of insulin from microspheres made from starch and carbopol, a reduced bioavailability and a lower decrease of blood glucose levels was noticed (Callens et al 2003a). The probable reason for this was the increase in viscosity of the nasal mucus, which would cause a physical barrier to absorption and strongly decelerate the mucociliary clearance.

*The ocular route*

Topical administration is the route of choice for treatment of ophthalmic diseases because of the blood–ocular barrier. Achieving therapeutic concentrations in the eye by systemic administration necessitates the usage of such high systemic concentration that, in many cases, systemic side effects and toxicity result. Delivering drugs to the eye is a challenge because there are several mechanisms that protect the eye from harmful materials and agents. These protective mechanisms are blinking, tear production and tear turnover. For drugs intended for locations within the eye, the passage through the corneal epithelium may impose problems because the cornea has a very tight epithelium. The cornea is an avascular structure with an epithelial layer that constitutes five or six layers of cells, of which the most superficial one consists of squamous cells (Figure 9). Underneath is an acellular layer called Bowman's membrane, followed by the stroma, which is a hydrated matrix (75–78% water) of collagen fibrils and glycosaminoglycans. The precorneal tear film consists of three layers – closest to the epithelium is an adsorbed

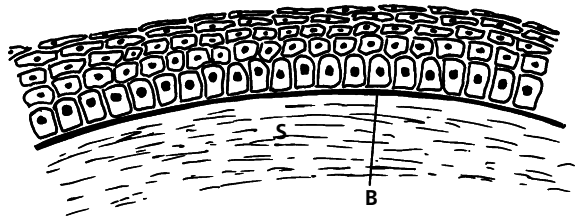


**Figure 9** Schematic drawing of the corneal epithelium overlaying Bowman's membrane (B) and the stroma (S).

mucin layer that act as a wetting agent, then comes the middle aqueous layer (the largest component of the tear film and some 6–10 $\mu$m thick) containing mucins with a higher concentration closer to the epithelium, and on the surface of the tear film there is a thin layer of lipid (0.1 $\mu$m). The mucin is predominantly produced by goblet cells found throughout the conjunctiva, which is a membrane of connective tissue covered by a multilayered epithelium continuous with that of the cornea. The conjunctiva covers the eye itself and the inner surface of the eyelids. Because of the limited volume in the precorneal area and the rapid tear turnover (16%/min), the normal contact time for water-based eye drops is approximately 5 min. To increase the residence time on the surface of the eye, viscosity-enhancing agents are often added. Other dosage forms used are suspensions and semisolid vehicles, such as gels and ointments, and ocular inserts.

Solutions containing different mucoadhesive polymers have been shown to increase the residence time in the eyes of rabbits (e.g. see Davies et al 1991; Durrani et al 1995; Felt et al 1999; Di Colo et al 2004). An increased residence time will increase the time over which absorption can occur and the total amount of drug absorbed, and has been shown to result in prolonged effect and increased bioavailability in several studies (Davies et al 1991; Burgalassi et al 1996; Herrero-Vanrell et al 2000).

The use of particulate dosage forms offers another possibility for prolonging the contact with the surface of the eye and also sustaining the release of the active substance. Chitosan microspheres have been used for delivery of aciclovir to rabbits resulting in an increase in both the duration and concentration of aciclovir in the aqueous humour in comparison with the values obtained with an aciclovir suspension (Genta et al 1997). Coating the microparticles with mucoadhesive polymers has also been evaluated as a potential way of increasing the bioavailability. Some examples are chitosan-coated nanocapsules, which increased the bioavailability of indometacin in rabbits (Calvo et al 1997) and PEG-coated nanospheres, which resulted in an increase in the bioavailability of aciclovir (Fresta et al 2001). Polymer coating of vesicles has also been found to reduce the drainage of the vesicles from the surface of the eye. For example, carbopol-coated vesicles reduced the drainage compared with non-coated vesicles, but the bioavailability of tropicamide (Davies et al 1992) and pilocarpine (Durrani et al 1992) was not affected. For chitosan-coated liposomes, on the other hand, an increased retention was not observed in rats (Henriksen et al 1996).

Polymeric inserts are the dosage forms that offer the longest residence times, and also offer several possible ways to control the release of the drug. The inserts can either be erodible or non-erodible, and can be used in the lower and sometimes in the upper conjunctival sac of the eye. Non-erodible silicone inserts grafted with a polymer network on the surface have been tested in rabbits (Chetoni et al 1998). The polymer-coated inserts were significantly better retained in the eye than uncoated inserts and the concentration of oxytetracycline, thus delivered, in the lachrymal fluid could be maintained at therapeutic levels for several days. Erodible inserts based on PEO have been investigated for the delivery of ofloxacin (Di Colo et al 2001a, b, 2002). The residence time in rabbit eyes was shown to be dependent on the molecular weight of PEO (Di Colo et al 2001a), and the bioavailability was 3–4 times and the $C_{max}$ 11–12 times the values obtained with commercial eye-drops. Addition of chitosan to the PEO inserts increased the concentration in the aqueous humour, probably as a result of enhanced corneal permeability (Di Colo et al 2002).

*The vaginal route*

Traditionally, this route of administration is used for delivering contraceptives and for local treatment, but it has also been explored for systemic delivery of drugs. In adults, the length of the vagina varies between 6 and 10 cm. The surface area is large owing to numerous folds and microridges in the epithelial layer. The epithelial layer consists of the lamina propria and a surface epithelium, which is a stratified squamous epithelium. The epithelial thickness varies with age – at birth it is thin, it thickens in puberty and becomes thin again after menopause. The thickness also varies during the menstruation cycle. Although there are no glands in the vaginal mucosa, the surface is usually covered with vaginal fluid, which is a mixture of fluids from a number of sources. The major components are cervical mucus and vaginal fluid from the well-vascularised mucosa. The volume, viscosity and pH of the cervical mucus vary with age and during the menstrual cycle. In fertile women the fluid is acidic and has a pH of 4–5. Dosage forms used for this route are solutions, suspensions, gels, creams, ointments, foams, pessaries, tablets and vaginal inserts. The residence time of the formulation is normally relatively short owing to the self-cleansing action of the vagina, the secretion of mucus and the humid site of administration. The most commonly studied mucoadhesive formulations are gels, microparticles and vaginal tablets.

For semisolid formulations, the residence time in the human vagina has been studied using a $^{99m}$Tc-marker and gamma scintigraphy. In a study by Brown et al (1997), the spreading and retention of vaginal pessaries made from Witepsol and a polycarbophil-based formulation were investigated in post-menopausal women. In five out of six subjects, the intra-subject variation was small, but there was a large inter-subject variation. The retention of the marker varied from 2% to 80% after 6 h, with no significant difference between the two formulations. A similar study using a cream and a gel formulation was made in pre-menopausal women (Chatterton et al 2004), with a similar result. There was no significant difference between the formulations and only a relatively small variation within individuals, but there was a large inter-individual variation. After 24 h, between 1 and 81% of the marker was retained. Gels have also been tested for local treatment of bacterial vaginosis. When a twice daily oral treatment of metronidazole was compared with local delivery from semisolid formulations, a metronidazole gel and clindamycin vaginal cream (Ferris et al 1995) resulted in almost equivalent cure rates to oral treatment. In another study, an in-situ gel formulation based on poloxamer was investigated as a vehicle for antifungal treatment in rats (Chang et al 2002) and it was concluded that the total dose given could be lowered by using a gel. In-situ gels have also been shown to be effective vehicles for immunisation in mice via the vaginal route (Oh et al 2003; Park et al 2003).

The retention of microspheres made from a benzyl ester of hyaluronic acid (HYAFF) was studied using gamma scintigraphy in sheep (Richardson et al 1996). The microspheres were either delivered as a dry powder or included in Suppocire $BS_2X$ pessaries. A substantial percentage of the radiolabelled spheres remained in the vagina at the end of the experiment (12 h after administration). The retention was greater for the dry powder formulation (85%) than for the pessary formulation (78%), which the authors suggested may be caused by loss of microspheres on leakage of the molten base. The HYAFF preparation was also used for delivery of salmon calcitonin (Richardson et al 1995), where the hypocalcaemic response was markedly enhanced by delivery in microspheres. In a different experiment, using bioadhesive starch microspheres, insulin was delivered vaginally in sheep (Richardson et al 1992). The authors compared the microspheres with an insulin solution, but the effect of a penetration enhancer, lysophosphatidylcholine (LPC), was found to be larger than the effect from the bioadhesive preparation.

In an efficacy study, mucoadhesive metronidazole vaginal tablets (100 mg metronidazole in a modified starch–PAA mixture) were compared with placebo and orally-delivered metronidazole ($2 \times 500$ mg daily) (Bouckaert et al 1995). There was a large difference between the placebo group and the two metronidazole groups, but no significant difference was found between the groups receiving vaginal tablets and oral metronidazole. In a later study, a cure rate of 64% was obtained with a single 100-mg dose given as a vaginal tablet (Voorspoels et al 2002), which was somewhat lower than the cure rate obtained for multiple applications of a gel formulation.

*The rectal route*

Rectal drug delivery is used both for local treatment and for systemic administration of drugs. The rectum is a part of the colon and forms the last 15–20 cm of the gastrointestinal tract; it has a surface area of approximately 300 $cm^2$. The epithelium consists of a single layer of cylindrical cells and goblet cells secreting mucus. The surface is relatively flat, without villi, and with only three major folds, the rectal valves. There is around 3 mL of mucus with a neutral pH

spread over the surface. Under normal circumstances the rectum is empty, and has a motility that enables spreading of even relatively viscous preparations. If absorption occurs in the lower part of the rectum, first-pass metabolism may be avoided. Dosage forms used are solutions, foams, gels and suppositories. However, not many studies have been performed using mucoadhesive rectal formulations.

Poloxamer, which is a temperature-sensitive in-situ gel, has been tested as a liquid suppository for several drugs (Choi et al 1998a, b; Kim et al 1998; Ryu et al 1999; Yong et al 2003). Addition of bioadhesive polymers has been made with the specific intention of increasing mucoadhesion and optimizing gel formation. The bioavailability of propranolol in rats increased with an increase in mucoadhesive force (Ryu et al 1999). Furthermore, the migration distance in the rectum decreased, which may be an important factor for drugs with an extensive first-pass metabolism. For the in-situ gelling formulation, a higher bioavailability of paracetamol was found in rats in comparison with the values obtained with conventional solid suppositories (Choi et al 1998a), but in man there was no significant difference in AUC (Kim et al 1998) even though a faster $T_{max}$ and a higher maximum plasma concentration was observed.

Addition of mucoadhesive polymers to conventional solid suppositories has also been made with the intention of restricting drug absorption to the lower part of the rectum. For example, the bioavailability of lidocaine in rats was found to be increased when carbopol was added to the suppository (Yahagi et al 1999).

*Concluding summary*

A considerable amount of knowledge has been compiled over the years that mucoadhesion has been studied, but despite this, there is still a need for further investigation. Given the different routes of administration together with their protective mechanisms, and the large variation in properties of the different dosage forms, there is no single answer to how a mucoadhesive formulation should be designed. The residence time of the formulation depends on the strength of the regions of the mucoadhesive complex and the weakest region will be where the failure occurs. When optimising the formulation, it is not sufficient to look only at the properties that affect interactions occurring in the interface, but one should also try to optimise the cohesive properties of the dosage form.

An increased knowledge has been gained about the properties that favour mucoadhesion but systemising the properties of importance for mucoadhesion are not easy, since the results obtained using different study methods can reflect different regions of the mucoadhesive complex. Some results found in the literature are related to the cohesion of the dosage form or the mucus, and in addition there is a time factor; during the contact with the mucosa, a change in the cohesion of both the dosage form and the mucus layer may have occurred as a result of water transport. Other methods are based on the assumption that certain characteristics, such as the interpenetration layer, are the most important feature for mucoadhesion, measuring only these characteristics. Given this variety in the methods used, and the fact that the mechanism responsible for the mucoadhesion can be dependent on the dosage form, the results found in the literature are not always unanimous.

Taken together, all of this makes mucoadhesion a complex subject. Irrespective of this there is no doubt that mucoadhesion is an important factor to consider when choosing a formulation and, if not for all, at least for several types of dosage forms, mucoadhesion will be an important factor for the duration of action and the bioavailability of drugs.

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