European Journal of Pharmaceutics and Biopharmaceutics 71 (2009) 505–518

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Review article

Mucoadhesive polymeric platforms for controlled drug delivery

Gavin P. Andrews *, Thomas P. Laverty, David S. Jones

School of Pharmacy, The Queen's University of Belfast, Belfast, Ireland

ARTICLE INFO

Article history:
Received 18 October 2007
Accepted in revised form 2 September 2008
Available online 18 October 2008

Keywords:
Bioadhesion
Mucoadhesion
Functional polymers
Drug delivery

ABSTRACT

The process of mucoadhesion involving a polymeric drug delivery platform is a complex one that includes wetting, adsorption and interpenetration of polymer chains amongst various other processes. The success and degree of mucoadhesion bonding is influenced by various polymer-based properties such as the degree of cross-linking, chain length and the presence of various functional groupings. The attractiveness of mucosal-targeted controlled drug delivery of active pharmaceutical ingredients (APIs), has led formulation scientists to engineer numerous polymeric systems for such tasks. Formulation scientists have at their disposal a range of *in vitro* and *in vivo* mucoadhesion testing setups in order to select candidate adhesive drug delivery platforms. As such, mucoadhesive systems have found wide use throughout many mucosal covered organelles for API delivery for local or systemic effect. Evolution of such mucoadhesive formulations has transgressed from first-generation charged hydrophilic polymer networks to more specific second-generation systems based on lectin, thiol and various other adhesive functional groups.

1. Adhesion, bioadhesion and mucoadhesion

Adhesives have been used extensively throughout human history, from early animal-derived pastes employed in prehistoric cave paintings to the use of super high-strength epoxy glues utilised in the manufacture of aircraft. Furthermore, adhesion processes play a very important role in nature having various healthcare implications; for example, bacterial adhesion and subsequent processes compromise the performance of many indwelling biomaterials often resulting in medical-device-related infection and hospital-acquired infection, increasing hospital costs by more than 500 million dollars annually [1]. Additionally, adhesion processes also affect non-biomedical areas: water purification, transport and storage systems acquire biofilms; whereas ship hulls and static marine structures are often colonized by marine organisms [2].

Adhesion as a process is simply defined as the "fixing" of two surfaces to one another [3]. There are many different terminological subsets of adhesion depending upon the environment in which the process occurs. When adhesion occurs in a biological setting it is often termed "bioadhesion", furthermore if this adhesion occurs on mucosal membranes it is termed "mucoadhesion". Bioadhesion can be defined as the binding of a natural or synthetic polymer to a biological substrate. When this substrate is a mucous layer, the term mucoadhesion is often used [4]. Mucoadhesion has been widely promoted as a way of achieving site-specific drug delivery through the incorporation of mucoadhesive hydrophilic polymers within pharmaceutical formulations along with the active pharmaceutical ingredient (API). The rationale being that the formulation will be 'held' on a biological surface for localised drug delivery. The API will be released close to the site of action with a consequent enhancement of bioavailability [5]. Whilst mucoadhesive drug delivery systems provide a means of enhancing retention at defined sites, if systemic uptake occurs the use of mucoadhesive polymers will not prevent a wider distribution of the API. Undoubtedly as a means of localising APIs to sites throughout the body, there are several advantages in using bio/mucoadhesive drug delivery systems:

(1) As a result of adhesion and intimate contact, the formulation stays longer at the delivery site improving API bioavailability using lower API concentrations for disease treatment.
(2) The use of specific bioadhesive molecules allows for possible targeting of particular sites or tissues, for example the gastrointestinal (GI) tract.
(3) Increased residence time combined with controlled API release may lead to lower administration frequency.
(4) The avoidance of first-pass metabolism.
(5) Additionally significant cost reductions may be achieved and dose-related side effects may be reduced due to API localisation at the disease site [5].

One of the earliest developed bioadhesive formulations was Orabase®. Orabase® was formulated from natural gums and represented the first purposely developed mucoadhesive. More recently,

* Corresponding author. School of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Ireland. Tel.: +44 2890 272646; fax: +44 2890 247794.
E-mail address: g.andrews@qub.ac.uk (G.P. Andrews).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.09.028

bioadhesive polymers have gained considerable interest through their use as auxiliary agents within peroral administration of peptide and protein drugs. The adhesive properties of such drug delivery platforms can reduce the enzymatic degradation due to the increased intimacy between the delivery vehicle and the absorbing membrane [6].

Over the last 30 years, the market share of transmucosal drug delivery systems has significantly increased with an estimated value of $6.7 million in 2006 [7]. According to a recent report published by Kalorama, worldwide revenue in this area is expected to increase approximately 3.5% a year to reach $7.9bn by 2010. This growth can be related to the ease with which transmucosal products may be designed and administered. For example, such dosage forms may be delivered via the nasal route using sprays, pumps and gels, via the oral/buccal route using mucoadhesives, quickly dissolvable tablets and solid lozenge formulations and via vaginal or urethral routes using suppositories, pessaries, vaginal rods and gels [7]. Furthermore, the sustained growth of biotechnology drugs and the inherent need for novel drug delivery technologies that provide easier and more controlled modes of administration has resulted in a dramatic increase in the use of transmucosal systems. In this respect, most targeted areas for mucoadhesive delivery systems centre on soft tissues that are normally covered in a layer of mucus derived from surrounding goblet cells or from specialised secretory glands.

2. Mucus: structure, function and composition

Mucus is a complex viscous adherent secretion which is synthesized by specialized goblet cells. These goblet cells are glandular columnar epithelium cells and line all organs that are exposed to the external environment. Mucus is found to serve many functions within these locations such as lubrication for the passage of objects, maintenance of a hydrated epithelium layer, a barrier function with regard to pathogens and noxious substances and as a permeable gel layer allowing for the exchange of gases and nutrients to and from underlying epithelium [8]. From an engineering point of view, mucus is an outstanding water-based lubricant whose properties are extensively exploited within nature. Giraffes can be seen using their sensitive mucus-laden tongues to strip the foliage from thorny acacia trees whilst slugs can crawl unharmed over a new razor blade [9].

Mucus is composed mainly of water (>95%) and mucins, which are glycoproteins of exceptionally high molecular weight (2–14×10^6 g/mol). Also found within this "viscoelastic soup" are proteins, lipids and mucopolysaccharides, which are found in smaller proportions (<1%). The mucin glycoproteins form 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. Furthermore, pendant sialic acid ($pK_a = 2.6$) and sulphate groups located on the glycoprotein molecules result in mucin behaving as an anionic polyelectrolyte at neutral pH [10].

Other non-mucin components of mucus include secretory IgA, lysozyme, lactoferrin, lipids, polysaccharides, and various other ionic species. Some of these non-mucin components are believed to be responsible for the bacteriostatic action observed in mucus [11]. Obviously, a thorough understanding of the glycoprotein mucin component is very important with regard to understanding the properties of mucus (Fig. 1). Mucin glycoproteins may be described as consisting of a basic unit made from a single-chain polypeptide backbone with two distinct regions [12]:

(1) A heavily glycosylated central protein core to which many large carbohydrate side chains are attached, predominantly via *O*-glycosidic linkages.

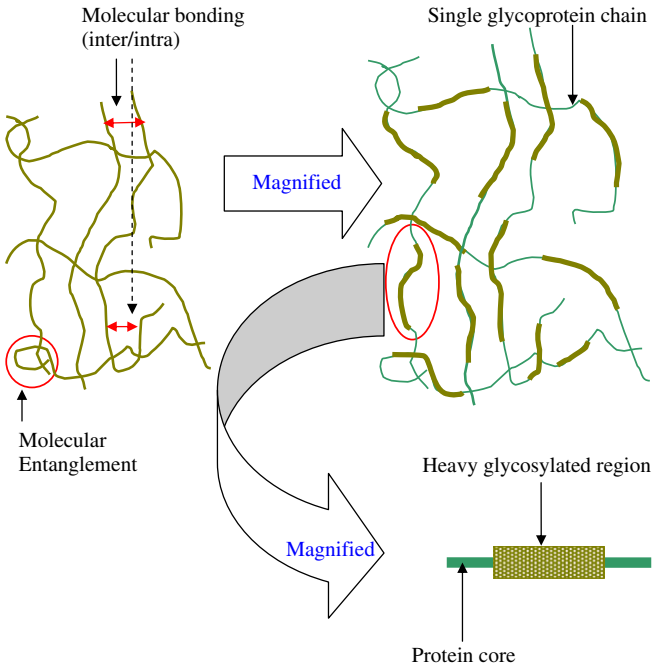

Fig. 1. The composition and interaction of glycoprotein chains within mucus.

(2) One or two terminal peptide regions where there is little glycosylation. These regions are often referred to as 'naked proteins regions'.

Mucin itself is stored in both submucosal and goblet cells, wherein the negative charges of the mucin glycoprotein are shielded by calcium ions, this allows for the compact packing of such molecules. During release into luminal space, outflux of calcium exposes these negative charges resulting in electrostatic repulsion and an approximate 400-fold expansion of the molecule. These now elongated mucin chains entangle and form non-covalent interactions such as hydrogen, electrostatic, and hydrophobic bonds leading to the development of a viscoelastic gel [13]. In the presence of water, these mucin chains begin to overlap, interpenetrate and form a structured network that mechanically functions as mucus. The overall rheological behaviour of mucus is a result of flow resistance exerted by individual chain segments, physical chain entanglement and non-covalent intermolecular interactions [14].

The exact composition of mucus may vary with the site of secretion, its physiological or mechanical role, and the presence of any underlying disease state [15]. One particular point of interest is the strategic position of mucus in many disease processes in which the interactions of epithelial cells and their surroundings have gone astray such as is seen in inflammatory and infectious diseases, cancer and metastasis [16]. Such scenarios may allow a means of targeting therapeutics to such conditions more effectively.

3. Mucoadhesion theories of polymer attachment

Mucoadhesion is a complex process and numerous theories have been presented to explain the mechanisms involved. These theories include mechanical-interlocking, electrostatic, diffusion–interpenetration, adsorption and fracture processes. Whilst undoubtedly the most widely accepted theories are founded surface energy thermodynamics and interpenetration/diffusion [17] These numerous theories should be considered as supplementary processes involved in the different stages of the mucus/substrate interaction, rather than individual and alternative theories [18].

3.1. The wettability theory

The wettability theory is mainly applicable to liquid or low viscosity mucoadhesive systems and is essentially a measure of the "spreadability" of the API delivery system across the biological substrate (Fig. 2). This theory postulates that the adhesive component penetrates surface irregularities, hardens and anchors itself to the surface. The adhesive performance of such elastoviscous liquids may be defined using wettability and spreadability; critical parameters that can be determined from solid surface contact angle measurements. This process defines the energy required to counter the surface tension at the interface between the two materials allowing for a good mucoadhesive spreading and coverage of the biological substrate [19]. Therefore the contact angle (ϕ), which may be easily determined experimentally, is related to interfacial tension (γ), of both components using

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG}\cos\phi \quad (1)$$

$$S = \gamma_{SG} - (\gamma_{SL} - \gamma_{LG}), \quad (2)$$

where γ_{LG} is liquid–gas surface tension, γ_{SL} is solid–liquid surface tension and γ_{SG} is solid–gas surface tension.

Mucoadhesive polymer systems that exhibit similar structure and functional groupings to the mucus layer will show increased miscibility; this in turn will result in a greater degree of polymer spreadability across the mucosal surface. Lower water: polymer contact angles of such systems will facilitate hydration of the polymer chains and thus promote intimate contact between polymeric delivery platform and the mucus substrate. In the case of an extremely hydrophilic polymer however, the water contact angle will be much lower than that of the mucosal surface, thus discouraging such an intimate contact due to a high interfacial surface free energy [20].

3.2. The electronic theory

This theory describes adhesion occurring by means of electron transfer between the mucus and the mucoadhesive system arising through differences in their electronic structures. The electron transfer between the mucus and the mucoadhesive results in the formation of a double layer of electrical charges at the mucus and mucoadhesive interface. The net result of such a process is the formation of attractive forces within this double layer [21]. Controversy has surrounded this theory arising from the statement that electrostatic forces are an important cause of bond adhesion, rather than merely a result of high joint strength [3].

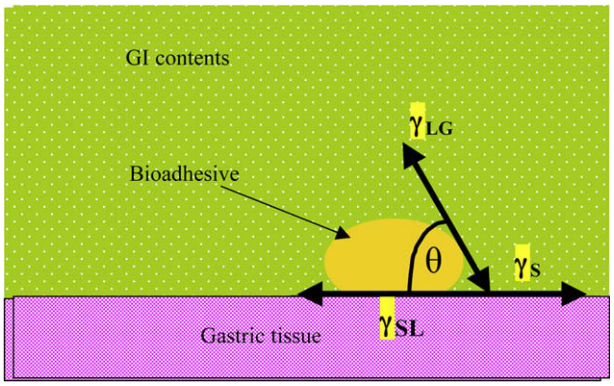

Fig. 2. The interfacial forces involved in polymer spreading, where θ is angle of contact, γ_{LG} is liquid–gas surface tension, γ_{SL} is solid–liquid surface tension, γ_{SG} is solid–gas surface tension.

3.3. The fracture theory

According to this theory, the adhesive bond between systems is related to the force required to separate both surfaces from one another. This "fracture theory" relates the force for polymer detachment from the mucus to the strength of their adhesive bond. The work fracture has been found to be greater when the polymer network strands are longer or if the degree of cross-linking within such as system is reduced [22]. This theory allows the determination of fracture strength (σ) following the separation of two surfaces via its relationship to Young's modulus of elasticity (E), the fracture energy (ε) and the critical crack length (L) through the following equation:

$$\sigma = \left(\frac{E \times \varepsilon}{L}\right)^{1/2} \quad (3)$$

[23]

3.4. The adsorption theory

In this instance, adhesion is defined as being the result of various surface interactions (primary and secondary bonding) between the adhesive polymer and mucus substrate. Primary bonds due to chemisorption result in adhesion due to ionic, covalent and metallic bonding, which is generally undesirable due to their permanency [3]. Secondary bonds arise mainly due to van der Waals forces, hydrophobic interactions and hydrogen bonding. Whilst these interactions require less energy to 'break' (Table 1) they are the most prominent form of surface interaction in mucoadhesion processes as they have the advantage of being semi-permanent bonds [3,24,22].

3.5. The diffusion-interlocking theory

This theory proposes the time-dependent diffusion of mucoadhesive polymer chains into the glycoprotein chain network of the mucus layer. This is a two-way diffusion process with penetration rate being dependent upon the diffusion coefficients of both interacting polymers (Fig. 3). Although there are many factors involved in such processes, the fundamental properties that significantly influence this inter-movement are molecular weight, cross-linking density, chain mobility/flexibility and expansion capacity of both networks [25]. Furthermore, temperature also has been noted as important environmental factor for this process [26].

Whilst it is acknowledged that longer polymer chains may diffuse, interpenetrate and ultimately entangle to a greater extent with surface mucus, it should be recognised that a critical chain length of at least 100,000 Da is necessary to obtain interpenetration and molecular entanglement. Additionally excessive chain cross-linking will act to decrease the polymer mobility and thus interfacial penetration [27]. Another significant contributory factor in determining interpenetration is the miscibility of both systems

Table 1
Typical bond types and energies, modified from Kinloch [3].

Type	Bond energy (kJ mol^{-1})
Primary bonding	
Ionic	590–1050
Covalent	63–710
Metallic	113–347
Secondary bonding	
Hydrogen bonding	10–42
Other dipole dipole	4–21
Dipole-induced dipole Deybe forces	<2
Dispersion (London) forces	0.08–42

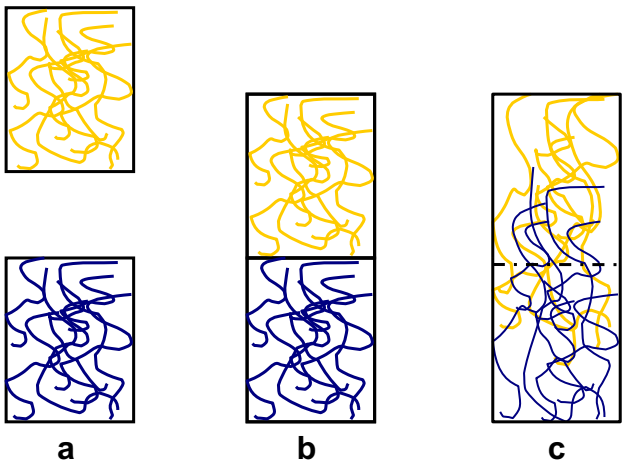

Fig. 3. The diffusion theory of adhesion. (a) Top (polymer) layer and bottom (mucus) layer before contact; (b) top layer and bottom layer immediately after contact; (c) top layer and bottom layer after contact for a period of time. Modified from Huang et al. [35].

with one another. It is reasonable to postulate then that maximum diffusion and bioadhesive strength may be achieved when the solubility parameter (δ) of the bioadhesive polymer and the mucus glycoprotein is similar [28]. The time at which maximum adhesion occurs between two substrates during interpenetration has been supported by experimental evidence in recent studies using AFT-FTIR and rheological techniques [17], and may be determined using the depth of interpenetration (I), and the diffusion coefficient (D_b),

$$t = \frac{I^2}{D_b} \quad (4)$$

[29]

4. Polymer properties affecting mucoadhesion

With reference to the theories of mucoadhesion, various polymer structural and functional groupings can have an effect on the likelihood and degree of polymer/mucus interaction. As such the potential for the modification or control of such polymer properties may allow for specific tailoring of mucoadhesive delivery systems.

4.1. Functional group contribution

The attachment and bonding of bioadhesive polymers to biological substrates occurs mainly through interpenetration followed by secondary non-covalent bonding between substrates. Given that secondary bonding mainly arises due to hydrogen bond formation, it is well accepted that mucoadhesive polymers possessing hydrophilic functional groups such as, carboxyl (COOH), hydroxyl (OH), amide (NH_2) and sulphate groups (SO_4H) may be more favourable in formulating targeted drug delivery platforms. Typically, physical entanglements and secondary interactions (hydrogen bonds) contribute to the formation of a strengthened network; therefore polymers that exhibit a high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins [17]. Mucoadhesive polymers are generally hydrophilic networks that contain numerous polar functional groups. Consequently, such functionalised polymers interact with the mucus not only through physical entanglements but also through secondary chemical bonds, thus resulting in the formation of weakly cross-linked networks [10]. The key sites for mucoadhesive interactions appear to be on the carbohydrate residues, via electrostatic interaction or through hydrophobic bonding of fucose clusters [30]. The significance of hydrogen bonding within mucoadhesion processes has been recently reported by Hagesaether and Sande [31]. Urea, a well-accepted hydrogen bonding disruptor significantly decreased the mucoadhesiveness of various mucus/pectin samples. The authors reported decreased cohesiveness and a loss of synergy within the combined pectin/mucin mixture.

4.2. Degree of hydration

Another important factor affecting the mucoadhesive strength of polymeric components is the degree of hydration. In this respect many polymers will exhibit adhesive properties under conditions where the amount of water is limited. However in such a situation, adhesion is thought to be a result of a combination of capillary attraction and osmotic forces between the dry polymer and the wet mucosal surface which act to dehydrate and strengthen the mucus layer [32]. Although this kind of "sticking" has been referred to as mucoadhesion, it is important to clearly distinguish such processes from "wet-on-wet" adhesion in which swollen mucoadhesive polymers attach to mucosal surfaces [33].Whilst hydration is essential for the relaxation and interpenetration of polymer chains, excess hydration could lead to decreased mucoadhesion and/or retention due to the formation of a slippery mucilage [34]. In this situation cross-linked polymers that only permit a certain degree of hydration may be advantageous for providing a prolonged mucoadhesive effect.

4.3. Polymer molecular weight, chain length, conformation, and degree of cross-linking

It is well accepted that structural polymeric components significantly influence the extent of diffusion, entanglement and hence mucoadhesion. A large molecular weight is essential for entanglement; however, excessively long polymer chains lose their ability to diffuse and interpenetrate mucosal surfaces [35]. Research within this field has shown that each polymeric system is unique preventing the definition of an optimum molecular weight. Dextrans, for example, with molecular weights of 19,500,000 and 200,000 possess similar bioadhesive strength which may be explained in terms of the helical conformation resulting in shielding of potential bioadhesive sites inside coiled conformers at higher molecular weights [23]). Conversely poly(acrylic) acid has an optimal MW of about 750,000, whereas polyethylene oxide has an optimum MW closer to 4,000,000 [36]. Whilst a critical length is necessary to produce bioadhesive interactions, additionally the size and shape of the interpenetrating polymeric chains must be considered [24,37].

The degree of cross-linking within a polymer system significantly influences chain mobility and resistance to dissolution. Cross-linked hydrophilic polymers swell in the presence of water allowing them to retain their structure, whereas similar high molecular weight linear hydrophilic polymers are swellable and readily dispersible. In mucoadhesive terms swelling is favourable as it not only allows greater control of drug release, but also additionally the swelling process increases the surface area for polymer/mucus interpenetration. As cross-link density increases, chain mobility decreases and hence the effective chain length, which can penetrate into the mucus layer decreases, reducing mucoadhesive strength [38]. Chain flexibility is critical for interpenetration and entanglement with the mucus gel. Increased chain mobility leads to increased inter-diffusion and interpenetration of the polymer within the mucus network [39].

4.4. pH and charge

The charge density of macromolecules is an important factor for bioadhesion with polyanions preferred to polycations when considering both toxicity and bioadhesion [40]. Macromolecular charge is affected by the pH of the physiological environment due to the dissociation of functional groups [29]. Undoubtedly there is the greatest potential for polymer mucus hydrogen bonding with undissociated anionic pendant functional groups. In relation to carboxylated polymers, pH values below the respective pK_a value would then be more favourable [41]. An article published almost 20 years ago by Park and Robinson [42] suggests that approximately 80% protonation of carboxyl groups is necessary for mucoadhesion within polyacrylic acid systems. This theory has been more recently discussed by Sudhakar et al. [38] with the suggestion that carboxylic groups in polyacrylic acids are only effective as interaction sites when in their acidic form. Whilst it is recognised that mucoadhesion processes are optimised in low pH environments, mucoadhesion may not be completely lost at higher pH values [43]. At higher pH levels, repulsion of "like" COO^- functional groups changes the spatial conformation from a coiled state into a "rod-like" structure making them more readily available for inter-diffusion and interpenetration [25]. Interestingly, above the pK_a of mucin a net negative charge may result in the repulsion of anionic species such as observed in ionised polyacrylic acid systems. At such elevated pH values,positively charged polymers, such as chitosan, may form polyelectrolyte complexes with negatively charged mucins and exhibit strong mucoadhesion [44].

Mucoadhesive polymers may be divided into three main groups in terms of overall charge, i.e., anionic, cationic and non-ionic systems. Anionic polymer systems such as the polyacrylic acids make up the bulk of pharmaceutically employed mucoadhesive polymers. The effect of polymer charge on mucoadhesion has been clearly demonstrated by Bernkop-Schnürch and Freudl [45]. In this work, various chemical entities were attached to chitosan and the mucoadhesiveness of the resulting charged polymer was assessed. Cationic chitosan HCl showed marked adhesiveness when compared to the control (a force of detachment of 32.4 ± 14.5 mN compared to 1.3 ± 0.1 mN). The attachment of ethylene diamine tetra acetic acid (EDTA), an anionic functional group, significantly increased mucoadhesive strength (81.7 ± 9.9 mN). Interestingly the addition of diethylene triamine penta acetic acid (DTPA) resulted in a system exhibiting both anionic and cationic characters. Such a phenomenon resulted from a lower binding affinity of DTPA when compared to that of EDTA possibly arising from steric hindrance. Mucoadhesive testing showed that the DTPA/chitosan system exhibited significantly lower mucoadhesive strength (3.0 ± 1.3 mN) in comparison to the cationic chitosan and anionic EDTA/chitosan complexes. This difference was attributed to the reduction in overall charge density that would have been expected within the DTPA/chitosan complex.

4.5. Polymer concentration

Polymer concentration has also been shown to significantly influence the strength of mucoadhesion. Optimal polymer concentration is dependent on physical state of the delivery system, with observational differences between semisolid and solid-state platforms. In the semisolid state, an optimum concentration exists for each polymer beyond which reduced adhesion occurs because a lower number of polymer chains are available for interpenetration with mucus. On the other hand, solid dosage forms such as buccal tablets exhibit increased adhesive strength as the mucoadhesive polymer concentration increases [19].

5. Environmental and physiological factors

There are numerous environmental and physiological factors that will have a marked effect on the mucoadhesive strength of polymer systems. Most of these factors such as pH and the amount of fluid at the biologically targeted area have been discussed previously. One significant factor not mentioned thus far is the variable mucus turnover at the applied surface/site throughout the body. Undoubtedly the most critical aspect is the time required to replenish the mucus layer. Such a shedding process is paramount in the body's fight against pathogens and will eventually lead to the shedding and eventual excretion of even the most adhesive drug delivery systems. The maximum duration in which a mucoadhesive system may adhere to the mucosal tissue will therefore be limited by the turnover time of the mucus gel layer. For some mucosal tissues, where mucus turnover is relatively low (e.g. mouth or vagina), this may be of less critical importance. However, in areas of markedly high mucus turnover such as in the intestines, adherence time is probably not longer than a couple of hours [46].

Mucus gel layer viscosity can vary throughout the body, with variability increasing in certain disease states. Low mucus viscosity results in a weak, easily detachable polymer/mucus bond, making targeted drug delivery extremely difficult. In contrast an extremely viscous mucus layer, such as those thickened due to white blood cell DNA, dead cells and inflammatory mediators limits the degree of interpenetration and also increases the diffusion pathway through which the active agent must pass [19,32].

Furthermore, the ionic strength of the surrounding medium may also have a significant role in defining the mucoadhesive bond force. In general mucoadhesion strength is decreased in the presence of ions due to shielding of functional sites that are pertinent for adhesion processes and importantly, gel network expansion [23,47,48,27]. It is worth to note that this generalization is not always applicable, and indeed certain polymer systems such as gellan are dependent upon the presence of divalent cations for *in situ* gelation [49].

6. Mucoadhesive polymer drug delivery platforms

The polymeric attributes that are pertinent to high levels of retention at applied and targeted sites via mucoadhesive bonds include hydrophilicity, negative charge potential and the presence of hydrogen bond forming groups. Additionally, the surface free energy of the polymer should be adequate so that 'wetting' with the mucosal surface can be achieved. The polymer should also possess sufficient flexibility to penetrate the mucus network, be biocompatible, non-toxic and economically favourable [20]. The polymers that are commonly employed in the manufacture of mucoadhesive drug delivery platforms that adhere to mucin–epithelial surfaces may be conveniently divided into three broad categories as defined by Park and Robinson [40]:

(1) Polymers that become sticky when placed in aqueous media and owe their bioadhesion to stickiness.
(2) Polymers that adhere through non-specific, non-covalent interactions that are primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant).
(3) Polymers that bind to specific receptor sites on the cell surface.

6.1. Traditional non-specific first-generation mucoadhesive polymers

First-generation mucoadhesive polymers may be divided into three main subsets, namely:

(1) Anionic polymers,
(2) Cationic polymers,
(3) Non-ionic polymers.

Of these, anionic and cationic polymers have been shown to exhibit the greatest mucoadhesive strength [27]. Consequently, such charged polymeric systems will now be examined in more depth.

6.1.1. Anionic polymers

Anionic polymers are the most widely employed mucoadhesive polymers within pharmaceutical formulation due to their high mucoadhesive functionality and low toxicity. Such polymers are characterised by the presence of carboxyl and sulphate functional groups that give rise to a net overall negative charge at pH values exceeding the pK_a of the polymer. Typical examples include poly(-acrylic acid) (PAA) and its weakly cross-linked derivatives and sodium carboxymethylcellulose (NaCMC). PAA and NaCMC possess excellent mucoadhesive characteristics due to the formation of strong hydrogen bonding interactions with mucin [50].

Polycarbophil (Noveon®) and carbomer (Carbopol®), PAA derivatives have been studied extensively as mucoadhesive platforms for drug delivery to the GI tract [51,52]. Polycarbophil is insoluble in aqueous media but has a high swelling capacity under neutral pH conditions, permitting high levels of entanglement within the mucus layer. Polycarbophil is also reported to increase its mass 100 times in aqueous media at neutral pH [53]. Additionally the non-ionized carboxylic acid groups bind to the mucosal surfaces via hydrogen bonding interactions [27]. PAA polymers are available in a wide range of molecular weights, form transparent, easily modified gel networks, are non-irritant, non-toxic and are considered safe (GRAS (Generally Recognized As Safe) status) for oral use by the FDA [54]. Furthermore, gel formation in such platforms is well understood, occurring as a result of electrostatic repulsion between anionic groups [55]. One clear distinction between carbomer and polycarbophil is the level of cross-linking and the cross-linking agent itself. Carbomers are cross-linked with allyl sucrose or allylpentaerythritol, whereas polycarbophil polymers are cross-linked with divinyl glycol. Both compounds have the same acrylic backbone but vary in their cross-link density that is often tailored to suit pharmaceutical and/or cosmetic performance.

6.1.2. Cationic polymers

Of the cationic polymer systems, undoubtedly chitosan is the most extensively investigated within the current scientific literature. Chitosan is a cationic polysaccharide, produced by the deacetylation of chitin, the most abundant polysaccharide in the world, next to cellulose [56]. The intriguing properties of chitosan have been known for many years with many examples of its use in agriculture, industry and medicine. Agriculturally, chitosan has been utilised as an antipathogenic [57], and from an industrial standpoint investigated as a metal-recovering agent [58]. Chitosan has been noted for its film-forming properties and has used extensively in cosmetics. Furthermore, chitosan has been employed as a dye-binder for textiles, a strengthening additive in paper and as a hypolipidic material in diets [59]. Among presently explored mucoadhesive polymers, chitosan is gaining increasing importance due to its good biocompatibility, biodegradability and due to their favourable toxicological properties [60]. Whereas PAAs bind to mucus via hydrogen bonds chitosan has been reported to bind via ionic interactions between primary amino functional groups and the sialic acid and sulphonic acid substructures of mucus [61–63]. Additionally, the hydroxyl and amino groups may interact with mucus via hydrogen bonding. The linearity of chitosan molecules also ensures sufficient chain flexibility for interpenetration [64]. Whilst chitosan may provide improved drug delivery via a mucoadhesive mechanism, it has also been shown to enhance drug absorption via the paracellular route through neutralisation of fixed anionic sites within the tight junctions between mucosal cells [65,66]. As previously discussed, chitosan is derived via the deacetylation of the naturally occurring, insoluble precursor chitin. Depending on the origin, chitin will generally become soluble in an aqueous acidic media when the degree of deacetylation exceeds 50%. This increase in solubility in an aqueous media is as a result of the protonation of the $-NH_2$ function on the C-2 position of the D-glucosamine repeat unit [67].

The major benefit of using chitosan within pharmaceutical applications has been the ease with which various chemical groups may be added, in particular to the C-2 position allowing for the formation of novel polymers with added functionality. Using such modifications, the properties of chitosan may be tailored to suit the requirements of specific pharmaceutical–technological challenges [68]. Work by Onishi and Machida [69] has demonstrated that chitosan and its degradation products are quickly eliminated by the kidney following intraperitoneal administration to mice, thus overcoming accumulation in the body.

6.2. Novel second-generation mucoadhesives

The major disadvantage in using traditional non-specific mucoadhesive systems (first generation) is that adhesion may occur at sites other than those intended. A scenario that is particularly true for platforms designed to adhere to a distal target such as those hypothesised in targeted mucoadhesion within the GI tract. Unlike first-generation non-specific platforms, certain second-generation polymer platforms are less susceptible to mucus turnover rates, with some species binding directly to mucosal surfaces; more accurately termed "cytoadhesives". Furthermore as surface carbohydrate and protein composition at potential target sites vary regionally, more accurate drug delivery may be achievable.

6.2.1. Lectins

Lectins are naturally occurring proteins that play a fundamental role in biological recognition phenomena involving cells and proteins (Table 2). For example, some bacteria use lectins to attach themselves to the cells of the host organism during infection. Enhancement of mucosal delivery may be obtained through the use of appropriate cytoadhesives that can bind to mucosal surfaces. The most widely investigated of such systems in this respect are lectins. Lectins belong to a group of structurally diverse proteins and glycoproteins that can bind reversibly to specific carbohydrate residues [70]. After initial mucosal cell-binding, lectins can either remain on the cell surface or in the case of receptor-mediated adhesion possibly become internalised via a process of endocytosis [46]. Such systems could offer duality of function in that lectin-based platforms could not only allow targeted specific attachment but additionally offer a method of controlled drug delivery of macromolecular pharmaceuticals via active cell-mediated drug uptake [46]. Whilst lectins offer significant advantages in comparison to first-generation platforms, it is worth noting that such polymers suffer at least in part from premature inactivation by shed off mucus. This phenomenon has been reported to be advantageous, given that the mucus layer provides an initial yet fully reversible binding site followed by distribution of lectin-mediated drug delivery systems to the cell layer [71]. Although lectins offer significant advantages in relation to site targeting, many are toxic or immunogenic, and the effects of repeated lectin exposure are largely unknown. It is also feasible that lectin-induced antibodies could block subsequent adhesive interactions between mucosal epithelial cell surfaces and lectin delivery vehicles. Moreover, such antibodies may also render individuals susceptible to systemic anaphylaxis on subsequent exposure [70].

Table 2
Principal function of lectins in nature. Modified from Ponchel and Irache [138].

Type	Function
Plants	Defence against phytopathogens Mediators of symbiosis Protection against predators (animals and insects) Storage proteins
Animals	Apoptosis Binding of bacteria to epithelial cells Defence against microorganisms Endocytosis and translocation of glycoproteins Regulation of cell migration and adhesion Recognition determinants in phagocytosis
Microorganisms	Attachment to host cells Recognition determinants in phagocytosis Recognition determinants in cell adhesion

6.2.2. Bacterial adhesions

Pathogenic bacteria readily adhere to mucosal membranes in the gastrointestinal tract, a phenomenon that has been exploited as a means by which target-specific drug delivery may be achieved. K99-fimbriae, an attachment protein derived from *E. coli*, has been covalently attached to polyacrylic acid networks [72]. The formulated polymer–fimbriae platform exhibited a significant increase in adhesion *in vitro* in comparison to the control (unmodified polymer).

6.2.3. Thiolated polymers

Thiolated polymers (thiomers) are a type of second-generation mucoadhesive derived from hydrophilic polymers such as polyacrylates, chitosan or deacetylated gellan gum [73]. Table 3 lists typical hydrophilic polymers that have been thiolated and the subsequent effect on mucoadhesive bond strength. The presence of thiol groups allows the formation of covalent bonds with cysteine-rich sub domains of the mucus gel layer, leading to increased residence time and improved bioavailability [74]. In this respect thiomers mimic the natural mechanism of secreted mucus glycoproteins that are also covalently anchored in the mucus layer by the formation of disulphide bonds [75,66]. Whilst first-generation mucoadhesive platforms are facilitated via non-covalent secondary interactions, the covalent bonding mechanisms involved in second-generation systems lead to interactions that are less susceptible to changes in ionic strength and/or the pH [76]. Moreover the presence of disulphide bonds may significantly alter the mechanism of drug release from the delivery system due to increased rigidity and cross-linking. In such platforms a diffusion-controlled drug release mechanism is more typical, whereas in first-generation polymers anomalous transport of API into bulk solution is more common [77].

Table 3
An example of thiolated polymers and the effect on measured mucoadhesion. Modified from Bernkop-Schnürch et al. [62].

Polymer	Mucoadhesive bond strength
Chitosan–iminothiolane	250-fold improved mucoadhesive properties
Poly(acrylic acid)–cysteine	100-fold improved mucoadhesive properties
Poly(acrylic acid)–homocysteine	Approximately 20-fold improved mucoadhesive properties
Chitosan–thioglycolic acid	Tenfold improved mucoadhesive properties
Chitosan–thioethylamidine	Ninefold improved mucoadhesive properties
Alginate–cysteine	Fourfold improved mucoadhesive properties
Poly(methacrylic acid)–cysteine	Improved cohesive and mucoadhesive properties
Sodium carboxymethylcellulose–cysteine	Improved mucoadhesive properties

7. Common sites of application for engineered mucoadhesive drug delivery platforms

The use of mucoadhesive formulations has been widely exploited for their targeted and controlled release delivery to many mucosal membrane-based organelles. Such formulations may deliver API for local or systemic effect, whilst bioavailability limiting effects such as enzymatic or hepatic degradation can be avoided or minimised.

7.1. Buccal drug delivery

The buccal cavity offers many advantages for drug delivery application, the most pertinent being high accessibility and low enzymatic activity. Additionally, buccal drug delivery can be promptly terminated in cases of toxicity through the removal of dosage form thereby offering a safe and easy method of drug utilisation [78]. Whilst first-generation mucoadhesives, such as sodium carboxymethylcellulose, hydroxypropylcellulose and polycarbophil [79], have been extensively examined, particularly for the treatment of periodontal disease [80,81], more recent investigations have focused on the controlled delivery of macromolecular therapeutic agents, such as peptides, proteins and polysaccharides [82]. Although gel and ointments are the most patient convenient; tablets, patches and films have also been examined [83]. Drug delivery to accessible cutaneous sites such as the buccal cavity is often associated with high patient compliance, low levels of irritation and offers significant ease of administration. Other less reported advantages include rapid onset of action due to a highly vascularised buccal mucosa and avoidance of hepatic first-pass metabolism [84].

Orabase®, a first-generation mucoadhesive paste, has long been used as barrier system for mouth ulcers. More recently, formulation development has resulted in a combined corticosteroid (triamcinolone acetonide) Orabase® product (Adcortyl in Orabase®), that provides local relief of mouth ulcers via a twofold mechanism; a barrier function and an anti-inflammatory function (due to triamcinolone acetonide). Although semisolid systems offer ease of administration and comfort [80], tablets and patches typically offer greater active ingredient stability (typically solid state), improved residence time and hence may provide longer periods of therapeutic drug levels at diseased sites. Commonly engineered tablet and patch platforms have included matrix devices and/or multilayer systems, containing an adhesive layer and other drug functional layers [78,85,79]. A drug impermeable layer is often included in such systems in order to encourage unidirectional drug release thus avoiding salivary gland clearance mechanisms. A common approach to avoid clearance of a tablet from the buccal cavity is to place the dosage form under the upper lip. Buccastem® an adhesive antiemetic tablet containing prochlorperazine maleate is administered in this way. Despite the advantages of bioadhesive tablets, the oscillatory action of talking and mastication can mean that some patients may find the use of such drug delivery platforms uncomfortable. This is one of the principal factors for the dominance of semisolid and flexible patch-based systems in buccal drug delivery.

7.2. Ophthalmic applications

The delivery of therapeutic agents to the eye may be achieved using various types of dosage forms including liquid drops, gels, ointments and solid ocular inserts (both degradable and non-degradable) [86–88]. Another interesting delivery platform is *in situ* gelling polymer that undergoes a phase transition after application. Pre-application these systems are in the liquid state

and are easily administered, whereas post-application they are transformed in highly viscous rheologically structured networks [89]. Transitional stimuli include temperature, pH, and the presence of certain ions (calcium ions) within the ocular fluid. One of the major concerns regarding the use of mucoadhesive polymers within the eye is the non-specificity of first-generation platforms. Mucoadhesive polymers would be expected only to attach to conjunctival mucus *in vivo*, but migration may result in causing deposition of semisolid within the corneal area, bringing with it a detrimental effect on visual acuity [25]. Additionally limited bioavailability has been experienced *in vivo* for carbomer and polycarbophil as a result of the high swelling capacity of such polymers in the neutral pH environment of the eye. Maintenance of a low viscosity in such systems through pH regulation in the range 4–5 is not acceptable as it may result in patient unease and mild lacrimation, both of which will have an effect on treatment success [53]. Further consideration should also be given to normal ocular clearance mechanisms (blinking) as well as lacrimation, both of which will enhance leakage from the applied site.

Undoubtedly the most common dosage form for application at this site is ophthalmic solutions. Interestingly, such drug delivery platforms typically exhibit poor bioavailability and therapeutic response because high tear fluid turnover results in rapid precorneal elimination of the active agent [90]. Consequently, high-frequency dosing is required and patient non-compliance is a major concern. Conversely, drug-loaded ocular inserts may offer improved control of drug release rate and longer residence times; however, disintegration into smaller pieces can result in occasional blurring of vision [91]. Furthermore, the rigidity of ophthalmic inserts is often extremely uncomfortable for patients. User acceptance and compliance may subsequently be limited by physical and psychological barriers surrounding such dosage forms [86].

7.3. Vaginal drug delivery systems

Vaginal drug delivery offers many advantages; the avoidance of hepatic first-pass metabolism, a reduction in the incidence and severity of gastrointestinal side effects, a decrease in hepatic side effects and avoidance of pain, tissue damage, and infection commonly observed for parenteral drug delivery routes of administration [92]. Whilst the vagina provides a promising site for systemic drug delivery because of its large surface area, rich blood supply and high permeability, poor retention due to the self-cleansing action of the vaginal tract is often problematic [93]. However, residence times within the vagina tend to be much higher than at other absorption sites such as the rectum or intestinal mucosa. Another important consideration is the change in the vaginal membrane during the menstrual cycle and post-menopausal period [94]. Furthermore, cultural sensitivity, personal hygiene, gender specificity, local irritation and influence of sexual intercourse are significant in determining the performance and success of the applied dosage form. Additionally, considerable variability in the rate and extent of absorption of vaginally administered drugs is observed by changes in thickness of vaginal epithelium [95].

Typical bioadhesive polymers that have been in vaginal formulations include polycarbophil, hydroxypropylcellulose and polyacrylic acid [95]. Although the major challenge for vaginal formulations is maximising coverage *in vivo* whilst minimising leakage [96], other important factors such as ease of use, absence of odour and lack of colour have been shown to significantly influence formulation acceptability [97]. There are several marketed formulations currently available, but undoubtedly the most difficult challenge is to prevent vaginal leakage. ACIDFORM®, a buffered mucoadhesive gel, has been shown to exhibit a greater intra-vaginal retention than other similar products (Conceptrol®, Advantage S®, Replens®, Aci-Jel® and K-Y jelly®). Moreover, after dilution with vaginal fluids and semen, ACIDFORM retained its viscoelasticity to a greater extent [98]. More recently ACIDFORM® has been shown to be present intra-vaginally 12 h after insertion [99]. Whilst mucoadhesive polymeric platforms provide longevity within the vagina it is extremely important particularly when designing drug delivery systems for the prevention of sexually transmitted disease to avoid mucosal irritation and damage of the epithelium; one of the natural protective barriers to disease. Vaginal mucosal irritation will certainly increase the susceptibility to sexually transmitted pathogens during sexual intercourse [100].

Although a large number of studies have been conducted to examine the potential of mucoadhesive polymer systems for the prevention and treatment of sexually transmitted diseases, the delivery of active agents for systemic delivery is also viable using such platforms. Oral bromocriptine used in the treatment of hyperprolactinemia, gives rise to a high proportion of gastrointestinal side effects. Therefore alternative routes of delivery with a much lower occurrence of side effects would be highly beneficial. More recently research has focused on the placement of commercial tablets in the vagina as a logical alternative for patients who cannot tolerate oral treatment. Many studies have demonstrated the superiority of the vaginal placement over the oral route in terms of dramatic minimisation of general and gastrointestinal side effects [101].

7.4. Nasal

From a histological point of view, the nasal mucosa provides an attractive route for systemic drug delivery. The total area of the human nasal mucosa is about 150 cm^2, which is surrounded by a dense vascular network, thus providing an excellent absorptive interface [23]. The nasal epithelium exhibits a relatively high permeability, with only two cell layers separating the nasal lumen from the dense vasculature within the lamina propria. Such factors make the nasal cavity an attractive route for drug delivery, but they also result in nasal mucosa cells being vulnerable to adverse effects of drugs and excipients delivered intranasally ([102]. One of the key advantages provided by intranasal drug delivery is that the nasal cavity provides a large highly vascularised surface area through which first-pass metabolism can be avoided, as blood is drained directly from the nose into the systemic circulation [65].

Successful nasal delivery has been obtained using solutions, powders, gels and microparticles. The most commonly employed intranasal APIs are solutions containing sympathomimetic vasoconstrictors for immediate relief of nasal congestion. Local delivery of these alpha adrenergic stimulators is of particular benefit to patients with high blood pressure (or those at heightened risk of cardiovascular incident), as vasoconstriction will occur to the greatest degree within the nose. In addition to local effects, the intranasal route of drug administration has also been used to achieve a distal systemic effect [103]. One such example is the intranasal delivery of the peptide desmopressin that exerts its action on the kidneys, mimicking the action of antidiuretic hormone, used mainly in Diabetes insipidus. Other such formulations include Imigran® (sumatriptan) and Miacalcic® (Calcitonin) nasal sprays that are used in the treatment of acute migrane and post-menopausal osteoporosis, respectively. It has also been shown that transnasal administration of large number of drugs (gentamicin, nafarelin acetate and ergotamine tartarate) results in blood levels comparable to intravenous delivery [104].

Whilst such delivery vehicles offer ease of administration, they suffer from a number of disadvantages, the most notable being rapid clearance from the nasal cavity thus preventing extended periods for drug release. Polymeric components such as hydroxypropylcellulose (HPC), chitosan, carbomer, NaCMC, hyaluronic acid and polyacrylic acid have all shown promise as mucoad-

hesive agents for use in controlled drug delivery to pulmonary and nasal sites. Such polymeric delivery platforms may be used either alone or as synergistic combination systems [105–108]. Poloxamers and polyethylene oxide have also found use in drug delivery to this region [109].

One of the most interesting areas of research within this field has been the use of intranasal drug delivery for the induction of antibody responses in serum, as well as local and distal mucosal secretions [109], due to absorption through the nasal-associated lymphoid tissue (NALT). In this respect a large body of research has been conducted using microparticulate systems [110–113]. Whilst inhaled particulate systems impacting on the mucus layer may be cleared rapidly by ciliary motion, they may also be selectively delivered to the organised NALT structures via the overlying specialised lymphoepithelium and induce an immune response [114]. Significant advantages in using such an approach include ease of administration and the generation of both systemic and mucosal immunities [5]. Despite the attractiveness of such a delivery pathway, there are certain problems that may arise through this type of drug delivery. Factors such as local tissue irritation, rapid mucociliary clearance, low permeability of the nasal membrane to larger macromolecules and the presence of proteolytic enzymes within intranasal cavity, may limit the full potential of API delivery in this way [115].

7.5. GI tract

Oral ingestion is the predominant and most preferable route for drug delivery. Delivery in this way allows for unassisted drug administration by the patient with the need for trained or skilled personnel being avoided. Such a situation is in contrast to what is experienced in most parenterally administered dosage forms [116]. Principally mucoadhesive polymers may offer increased intimacy with the lining of the GI tract and hence bioavailability [117]. Furthermore, "absorption windows" within the GI tract such as those making up the gastro-associated lymphatic tissue (GALT) may be targeted allowing for the absorption of larger poorly soluble therapeutic agents [118]. Despite a few notable exceptions, mucoadhesive drug delivery systems have to date not reached their full potential within oral drug delivery. This is simply attributed to insufficient adhesion within the GI tract to provide a prolonged residence time [62,119]. Targeted drug delivery systems in this respect have focused on mucoadhesive patches and microparticles using first-generation polymers [120]. The significant problem with large mucoadhesive solid dosage forms such as tablets is the poor adherence to mucosal surfaces due to large dosage form mass combined with the vigorous movement of the gastrointestinal tract.

Although first-generation polymers have had limited success, second-generation vehicles are now receiving increased attention. A thiolated chitosan tablet has recently been reported by Krauland et al. [121] for the oral delivery of insulin. Significantly decreased glucose levels in non-diabetic rats compared to unmodified polymer insulin tablets were reported. This was attributed to the presence of two enzyme inhibitors (chitosan and thiol groups), a penetration enhancing effect of the polymer system and the mucoadhesive potential of the system. Further advances in this field have included the attachment of second-generation mucoadhesives to the surface of microspheres [122]. Furthermore, Säkkinen et al. [123] have examined the potential of microcrystalline chitosan granules for the delivery of furosemide. Gamma scintigraphy revealed that gastrointestinal transit *in vivo* was extremely erratic, with adhesion to the gastric mucosa in only one-third of the granules administered. Although the use of mucoadhesive delivery platforms may result in altered pharmacokinetic profiles, there is limited evidence to suggest that such a phenomenon is directly related to increased adhesion within the gastrointestinal tract [118]. Although in principal such dosage forms may provide increased adhesion and thus improved bioavailability; peristalsis, high mucus turnover and encasement of the delivery vehicle within a mucus shell are significant factors limiting their success [118].

8. Measuring the mucoadhesive potential of candidate delivery platforms

Despite the intense focus surrounding mucoadhesive systems over the past few decades, no standard test methods have been specifically designed for mucoadhesion analysis. This places limitations upon the direct comparison of data obtained from different research groups. Despite this, researchers have continued to develop novel ways in which the adhesive potential of polymer systems may be ranked in relation to their adhesiveness. The majority of such testing techniques throughout the literature are found to be *in vitro* in nature. Despite this, there are a few *in vivo* experimental setups that have also been successfully implemented.

8.1. In vitro studies of mucoadhesion

In vitro tests are by far the most common techniques for the determination of polymers mucoadhesive properties [124]. There are various experimental setups currently in use throughout the scientific community, in recent years these tests have evolved from simple measurements of force of detachment to much more sophisticated and expensive setups.

8.1.1. Measuring the force of attachment

The most obvious method in which to assess a systems mucoadhesiveness is through the determination of the adhesive strength between polymer and the attached substrate. The adhesive strength at such a bonding interface can be measured by measuring the force required to detach one entity from the other through the application of an external force. As such the destruction of the adhesive bond is usually under the application of either a shearing, tensile or peeling force. [29]. The determination of such a force of attachment was investigated by Smart et al. [125]. Here the authors used a modified version of the Wilhelmy plate surface technique in order to determine mucoadhesion of a range of candidate polymers (Fig. 4). The device itself basically consists of a glass plate (which is laden with the polymer to be studied) suspended from a microbalance. The polymer-coated plate was then slowly dipped into a beaker of mucus. The work required to remove the various polymer-coated glass slides could then be related to one

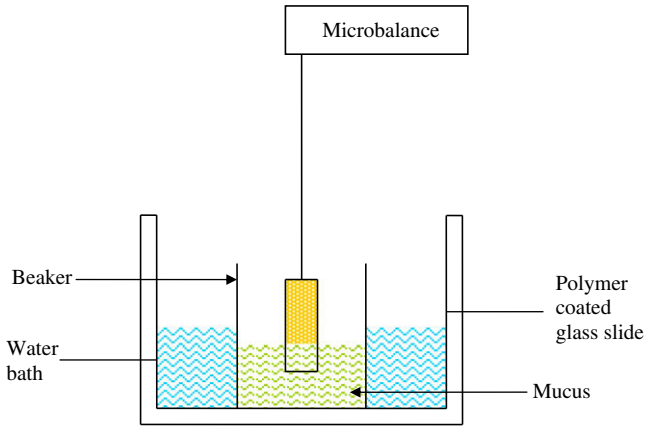

Fig. 4. Illustrating the modified Wilhelmy plate surface technique, for mucoadhesion determination. Modified from Smart et al. [125].

another and their adhesiveness could be ranked. This technique had the advantage of allowing the analysis of mucoadhesion under different environmental conditions via simple modification of instrumental setup. Subsequently though Mikkos and Peppas [29] pointed out the short comings of such a technique due to the possible dissolution of the polymer upon testing. They suggested that this effect may be limited if polymer plates of the candidate material were used instead of polymer-coated glass plates. Further shortcomings were also detailed by Wong et al. [126] who noted that the lack of biological tissue in such a setup may not represent true mucoadhesion. Despite the simplicity and efficiency of such a technique, tensile tests provide an incomplete picture of the process of mucoadhesion. It must be borne in mind that most mucoadhesive delivery systems will tend to exhibit other mechanical forces, such as the shear stresses exhibited within the buccal cavity or GI tract [127,128]. The effect of both these important forces on measuring the adhesive bond was made possible via the use of a dual tensiometer such as the one used by Leung and Robinson [127] (Fig. 5).

Despite the proposed advantages of such a technique, more recent work has in fact been based on tensile-based setups. In particular the use of modified textural analysers has been extensively studied. In the mid 1990s, Tobyn et al. [129] validated a new technique of mucoadhesion testing using a TA-XT2 texture analyser and porcine stomach tissue. Further work by Wong and co-workers [126] closely looked at the effect of various instrumental parameters on candidate mucoadhesive polymers. Their findings indicated that the mucoadhesive determination of polymers could be influenced by variables such as contact force, contact time and the speed of probe removal from the mucosal tissue. A longer contact time and higher probe speed were found to give a greater degree of sensitivity arising from greater reproducible in results along with higher measurement values. In terms of the contact force, the authors determined that a certain level of force affected the mucoadhesion, beyond which further increases in force had little effect. In conclusion, the authors stated that both the work of adhesion and peak detachment force could be used in the evaluation of mucoadhesiveness of candidate adhesive polymer systems. Mortazavi and Smart [128] investigated the importance of the nature of the mucosal surface within an *in vitro* experiment; mucus gel, rat small intestine and a control of PVC tape (non-sticky side used) were all investigated. The authors determined the rank of adhesion as follows: PVC > Rat small intestine > mucus gel, indicating that a specific mucus/mucoadhesion interaction is not paramount in the adhesive ranking of these polymers. The apparatus used within the study had the potential to measure tensile and shear forces and was similar to the one employed by Leung and Robinson [127]. In fact the difficulty the authors had in measuring shear stresses within the study could point to why the use of such a technique has been limited to date.

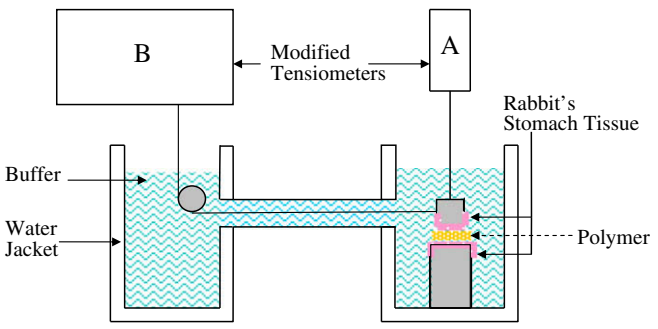

Fig. 5. Illustrating the modified dual tensiometer used by Leung and Robinson [127].

A method more recently employed by Grabovac et al. [130] does in fact allow for the measurement of mucoadhesion under the application of a shearing stress. The test is centred on the use of a rotating cylinder, wherein the adhesion time of various compressed polymer discs to porcine small intestinal mucosa was established. The procedure itself involved attaching polymer discs to freshly excised porcine intestinal mucosa which had prior been spanned onto a stainless steel cylinder. The cylinder was then placed within a dissolution apparatus and rotated at a speed of 125 rpm. Changes in the test system were visually determined every 30 min and measured until all the discs were either disintegrated or detached from the mucosal surface.

8.1.2. Rheological measurement of mucoadhesion

Numerous authors have suggested that the rheological profiling of polymer–mucus mixtures can provide an acceptable *in vitro* model representative of the true *in vivo* behaviour of a mucoadhesive polymer [41]. The initial implementation of such rheological techniques was initially suggested by Hassan and Gallo [63]. Within this study the mucoadhesive potential of polymer candidates was determined by rheologically comparing binary polymer/mucus blends to the rheological sum of similarly concentrated mono component mucus and polymer systems. Findings showed that the mucoadhesive polymer/mucus mixtures exhibited synergistic rheological profiles, the causes of which were attributed to bond formation between polymer and mucus culminating in an increase in total system structure. Since this pioneering work, there have been numerous rheological studies of polymer/mucus interactions.

Madsen and co-workers [131] undertook work that indicated that rheological synergism was found to arise only within a certain polymer concentration range and that this concentration was dependent upon the polymer under study. Furthermore authors discussed the benefit of using non-destructive dynamic rheological analysis to measure the interaction parameters as opposed to destructive flow technique that had been previously implemented. In an extension to this work, Madsen and colleagues [17] determined the interactions between four mucoadhesive polymers (Noveon®, Pemulen® TR-2, carageenan and sodium carboxymethylcellulose) and a homogenised mucus gel. Using a dynamic rheological technique they showed that incorporation of a mucoadhesive polymer into mucus gel produced rheological behaviour that was indicative of a weakly cross-linked gel network. In addition they noted that optimum gel strengthening occurred within a weakly acidic environment, reinforcing the idea that optimum conformation and degree of ionisation of the polymer and mucus molecules are important factors for mucoadhesion. In contrast to the other measured polymer systems, the study showed that carboxymethylcellulose/mucus mixes exhibited no synergy at all.

The validity of such rheological measurements as a method of ranking mucoadhesive order was reinforced by the work undertaken by Tamburic and Craig [132]. Within this study, the authors compared tensile mucoadhesive testing with shear rheological measurements using three polyacrylic acid-based polymers. They found that both measuring techniques gave the same rank order over adhesiveness for the polymers studied.

The isolation of fresh mucus for mucoadhesion analysis can be a laborious task. This has led many authors to turn their attention to the use of commercially dried mucus which is then re-hydrated before testing. Validation of the use of such mucus was carried out by Rossi et al. [133]. Within this study, investigators determined if there was in fact any variation in mucoadhesion testing through the use of different mucus-derived substrates. The authors rheologically studied the effect of two different freeze-dried porcine mucins and one type of freeze-dried bovine mucin on rheological parameters. Through their investigations, they discovered that

the choice of mucus type was important in particular for the ion-sensitive polyacrylic acids. Polyacrylic acids showed only positive interaction with the bovine-derived mucin, whereas the less ion-sensitive NaCMC had positive interaction with all tested mucus. The lack of interaction between polyacrylic acid and the porcine mucin was suggested to be due to the greater number of adhesion-impeding ions present within porcine mucin. In conclusion, authors stated that mucoadhesive polymers can be affected not only by the biochemical structure of glycoproteins but also by the way the mucin sample is prepared.

Later work by Kocevar-Nared and co-workers [14] again focused on the effect of mucus type on mucoadhesion testing. In this work comparison of re-hydrated dried crude porcine gastric mucin to that of freshly isolated mucus from the porcine gut was carried out. The study showed that no matter what loading of dried mucus was reconstituted; rheological properties similar to freshly isolated mucus could not be achieved. The authors concluded that the isolation procedures changed the physicochemical properties of the mucus to such an extent that reconstitution resulted on mucus of an altered structure. Despite experimental work showing that re-hydrated commercial mucus does not posses the exact viscoelastic structure of freshly isolated mucus, Hägerström et al. [32] pointed out that the use as of fresh mucus gels is not unquestionable since it requires reproducible isolation and purification procedures so that the amount of glycoproteins able to interact always remains the same.

8.1.3. Other in vitro tests

Despite the popularity within the literature of the force of mucoadhesive attachment determination and rheological-based techniques, there are other adhesive testing methods which may offer an alternative insight into the method and degree of mucoadhesion under suitable conditions.

Park and Robinson [40] determined the effect of various polymer and mucin interactions via the use of fluorescent probes. The technique involved labelling the lipid bilayer of cultured human conjunctiva cells with the fluorescent probe pyrene. The adhesion of polymers to these cells caused a change in fluorescence due to surface compression when compared to control cells. This degree of change in fluorescence is proportional to the amount of polymer binding. Furthermore, the use of another probe also allowed the effect of polymer charge, charge sign and density on adhesion to be determined. Their findings suggested that highly charged carboxylated anions exhibit the best properties for bioadhesive drug delivery systems. The benefit of such a technique was highlighted by Mikos and Peppas [29] who stated that such a technique appreciated the fact that the determination of the adhesive bond is based on molecular interactions. Despite this, these authors also pointed out the limitations of such a technique stating that such measurements did not take into account those macromolecules that bind perpendicularly.

The continual evolution of such *in vitro* techniques has been seen more recently in the work by Batchelor and co-workers [134]. Here fluorescently labelled alginate solutions of known rheological profile were delivered onto porcine oesophageal tissue. A washing solution was applied at a specified rate to mimic saliva flow, and the eluted material collected with the degree of retention over time measured via fluorimetric analysis. Investigators showed that after 30 min up to 20% of the applied alginate dose remained associated with the tissue, regardless of the type alginate selected.

An imaging technique that did not use fluorescently labelled polymers was derived by Kockisch and colleagues [135]. Here investigators developed a semi-quantitative image analysis-based technique for the *in vitro* and *in vivo* detections of polymers with an affinity for the mucosal surfaces of the oral cavity. This technique was used to analyse various well-known mucoadhesive polymers, allowing the visualisation of the adhering polymers to buccal cell scrapings. Visualisation of adhesion was aided through staining with 0.1% (w/v) of either Alcian blue (for polyanions) or Eosin (for chitosan) solution with uncomplexed dye being removed with 0.25 M sucrose washings. The extent of polymer adhesion was then quantified by measuring the relative staining intensity of control and polymer-treated cells by image analysis.

A completely different *in vitro* technique was carried out by Takeuchi and co-workers [136] who looked at the measurement of mucoadhesion of various adhesive polymers via the BIACORE instrumentation. This system is based on the principle underlying an optical phenomenon called Surface Plasmon Resonance (SPR). Such a system measures the refractive index, which varies with the solute content of a solution that comes in contact with the sensor chip. An SRP response is achieved when a molecule becomes attached to the surface of the sensor chip as the solute concentration on the chip increases. As such, quantitative measurements can be achieved via the binding interaction between the chip surface and one or more functional groups such as NH_2, SH, CHO and COOH. The procedure itself involved the immobilization of mucoadhesive polymer on the sensor chip surface with a mucin suspension being passed over the sensor chip for a predefined time. When the mucin particle binds to the polymer on the sensor chip surface, the increased response is measured; when they dissociate, the response will fall. Such an instrument setup allows for the real-time measurement and label-free detection of polymer mucin binding.

8.2. In vivo studies

Due to cost, time constraints and ethical considerations, *in vivo* mucoadhesive studies are less commonly seen in the literature than *in vitro* testing. Despite these concerns *in vivo* testing is still the important if the true mucoadhesive potential of a system is to be determined. As such *in vivo* techniques have found there most extensive use in the analysis of potential oral mucoadhesive dosage forms.

In 1985 Ch'ng et al. [43] studied the transit of various ^{51}Cr radio labelled polyacrylic acid beads through the rat GI tract. The beads were fed to the rats and at various time intervals after which the rats were sacrificed. The rat's intestine was then systematically dissected into 20 equal lengths and the amount of radiation in each section measured, thus allowing the transit overtime to be realised. Such an experimental technique obviously had its limitations due to the requirement for the sacrifice of the subject. The development of a non-invasive technique to determine the transit of mucoadhesive polymers was undertaken in the work by Davis [137]. Here, the transit of polymers could be imaged via labelling of the mucoadhesive polymer system with a γ-emitting nucleotide. Detection of the polymer systems within the GI tract was then determined via γ-scintigraphy.

Finally a more recently employed non-invasive imaging technique was presented by Albrecht et al. [74]. Investigators here used magnetic resonance imaging to localise the point of release of thiolated polymers from dosage forms via the use of gadolinium. *In vivo* mucoadhesion was determined by ascertaining the residence time of the fluorescently-tagged thiomer on intestinal mucosa of rats after 3 h. This technique allowed the comparison of mucoadhesive properties of candidate polymer systems for oral drug delivery *in vivo*.

9. Conclusions

The complex procedure of mucoadhesion can allow for the target-controlled delivery of a range of APIs. Certain polymer properties such as charge, hydrophilicity, molecular weight amongst

other parameters can affect the success and strength of adhesive bond. Furthermore, environmental factors such as the tonicity and mucus turnover rate must also be considered prior to formulation. Taking such considerations into account, polymers can be chemically structured and engineered to fit a particular pharmaceutical application. Despite the lack of a universal test for mucoadhesion, numerous techniques are available that allow for mucoadhesive ranking of polymer systems. Such systems are usually *in vitro* in nature due to their relative ease of implementation and cost-effectiveness and as such may present an efficient way of selecting candidate delivery systems for further more intensive *in vivo* testing. The most successful first-generation mucoadhesive polymer systems have been centred on hydrophilic, high molecular weight, anionic species such as carbomers. Such polymeric networks have found widespread use within the mucus-lined organelles of the nose, buccal cavity and the vagina to name but a few. Despite the controlled release of pharmaceutical actives such systems offer, the specific targeting of particular mucosal sites has fallen short. In particular the holy grail of mucoadhesive drug delivery has been centred around delayed transit and/or targeting of adhesive polymer drug delivery platforms to particular "absorption windows". Such a system could have numerous potential applications, not least improvement in the bioavailability of current poorly absorbed GI drugs, but to date results in the literature have been mixed. More recently attention has shifted away from these more traditional mucoadhesive polymers towards systems based on the new second-generation mucoadhesives. These second-generation mucoadhesives usually involve the attachment of lectin, thiol or various other adhesin functional groupings to traditional first-generation polymers networks. As such the binding of these types of platforms offer, the possibility of controlled release and a greater degree of attachment specificity, perhaps even within the GI tract. Despite the promise of these advanced formulations more work is required, not least on toxicity profiling, before the true potential of such engineered formulations may be realised.

References

[1] M.E. Rupp, T. Fitzgerald, N. Marion, V. Helget, S. Puumala, J.R. Anderson, P.D. Fey, Effect of silver-coated urinary catheters: efficacy, cost-effectiveness, and antimicrobial resistance, Am. J. Infect. Control 32 (2004) 445–450.
[2] P. Kingshott, H. Griesser, Surfaces that resist bioadhesion, Curr. Opin. Solid State Mater. Sci. 4 (1999) 403–412.
[3] A.J. Kinloch, The science of adhesion, J. Mater. Sci. 15 (1980) 2141–2166.
[4] I. Henriksen, K. Green, J. Smart, G. Smistad, J. Karlsen, Bioadhesion of hydrated chitosans: an in vitro and in vivo study, Int. J. Pharm. 145 (1996) 231–240.
[5] J. Woodley, Bioadhesion: new possibilities for drug administration?, Clin Pharmacokinet. 40 (2001) 77–84.
[6] A. Bernkop-Schnürch, C. Humenberger, C. Valenta, Basic studies on bioadhesive delivery systems for peptide and protein drugs, Int. J. Pharm. 165 (1998) 217–225.
[7] Drug Delivery Markets. Vol. IV: Transdermal and Transmucosal Drug Delivery, Kalorama Information Report, 2007, KLI1399450.
[8] R. Bansil, B. Turner, Mucin structure, aggregation, physiological functions and biomedical applications, Curr. Opin. Colloid Interf. Sci. 11 (2006) 164–170.
[9] J. Davies, C. Viney, Water–mucin phases: conditions for mucus liquid crystallinity, Thermochim. Acta 315 (1998) 39–49.
[10] R. Capra, A. Baruzzi, L. Quinzani, M. Strumia, Rheological, dielectric and diffusion analysis of mucin/carbopol matrices used in amperometric biosensors, Sensors Actuators B 124 (2007) 466–476.
[11] A. Allen, D. Snary, The structure and function of gastric mucus, Gut 13 (1972) 666–672.
[12] I. Fiebrig, S. Harding, A. Rowe, S. Hyman, S. Davis, Transmission electron microscopy studies on pig gastric mucin and its interactions with chitosan, Carbohydr. Polym. 28 (1995) 239–244.
[13] R. Willits, W.M. Saltzman, Synthetic polymers alter the structure of cervical mucus, Biomaterials 22 (2001) 445–452.
[14] J. Kocevar-Nared, J. Kristl, J. Smid-Korbar, Comparative rheological investigation of crude gastric mucin and natural gastric mucus, Biomaterials 18 (1997) 677–681.
[15] K. Khanvilkar, M. Donovan, D. Flanagan, Drug transfer through mucus, Adv. Drug Delivery Rev. 48 (2001) 173–193.
[16] J. Dekker, J.A. Rossen, H. Büller, A.C. Einerhand, The MUC family: an obituary, Trends Biochem. Sci. 27 (2002) 126–131.
[17] F. Madsen, K. Eberth, J. Smart, A rheological assessment of the nature of interactions between mucoadhesive polymers and a homogenised mucus gel, Biomaterials 19 (1998) 1083–1092.
[18] H.E. Bodde, Principles of bioadhesion, in: R. Gurny, H.E. Junginger (Eds.), Bioadhesion – Possibilities and Future Trends, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1989, pp. 44–64.
[19] M.I. Ugwoke, R.U. Agu, N. Verbeke, R. Kinget, Nasal mucoadhesive drug delivery: background, applications, trends and future perspectives, Adv. Drug Deliv. Rev. 57 (2005) 1640–1665.
[20] A. Shojaei, X. Li, Mechanisms of buccal mucoadhesion of novel copolymers of acrylic acid and polyethylene glycol monomethylether monomethacrylate, J. Control. Release 47 (1997) 151–161.
[21] D. Dodou, P. Breedveld, P. Wieringa, Mucoadhesives in the gastrointestinal tract: revisiting the literature for novel applications, Eur. J. Pharm. Biopharm. 60 (2005) 1–16.
[22] A. Ahagon, A.N. Gent, Effect of interfacial bonding on the strength of adhesion, J. Polym. Sci. Polym. Phys. 13 (1975) 1285–1300.
[23] J.M. Gu, J.R. Robinson, S.H. Leung, Binding of acrylic polymers to mucin/epithelial surfaces: structure–property relationships, Crit. Rev. Ther. Drug Carrier Syst. 5 (1988) 21–67.
[24] M.R. Jiménez-Castellanos, H. Zia, C.T. Rhodes, Mucoadhesive drug delivery systems, Drug Dev. Ind. Pharm. 19 (1993) 143–194.
[25] J.W. Lee, J.H. Park, J.R. Robinson, Bioadhesive-based dosage forms: the next generation, J. Pharm. Sci. 89 (2000) 850–866.
[26] E. Jabbari, N.A. Peppas, A model for interdiffusion at interfaces of polymers with dissimilar physical properties, Polymer 36 (1995) 575–586.
[27] A. Ludwig, The use of mucoadhesive polymers in ocular drug delivery, Adv. Drug Deliv. Rev. 57 (2005) 1595–1639.
[28] J. Vasir, K. Tambwekar, S. Garg, Bioadhesive microspheres as a controlled drug delivery system, Int. J. Pharm. 255 (2003) 13–32.
[29] A.G. Mikos, N.A. Peppas, Systems for controlled release of drugs. V: Bioadhesive systems, STP Pharma. Sci. 19 (1986) 705–715.
[30] S.E. Harding, Trends in muco-adhesive analysis, Trends Food Sci. Technol. 17 (2006) 255–262.
[31] E. Hagesaether, S.A. Sande, In vitro measurements of mucoadhesive properties of six types of pectin, Drug Dev. Ind. Pharm. 33 (2007) 417–425.
[32] H. Hagerstrom, M. Paulsson, K. Edsman, Evaluation of mucoadhesion for two polyelectrolyte gels in simulated physiological conditions using a rheological method, Eur. J. Pharm. Sci. 9 (2000) 301–309.
[33] H. Sigurdsson, T. Loftsson, C. Lehr, Assessment of mucoadhesion by a resonant mirror biosensor, Int. J. Pharm. 325 (2006) 75–81.
[34] S.A. Mortazavi, J. Smart, An investigation into the role of water movement and mucus gel dehydration in mucoadhesion, J. Control. Release 25 (1993) 197–203.
[35] Y. Huang, W. Leobandung, A. Foss, N.A. Peppas, Molecular aspects of muco- and bioadhesion: tethered structures and site-specific surfaces, J. Control. Release 65 (2000) 63–71.
[36] X. Yang, J.R. Robinson, Bioadhesion in mucosal drug delivery, in: T. Okano (Ed.), Biorelated Polymers and Gels Controlled Release Applications in Biomedicine, Academic Press, San Deigo, 1998.
[37] S.A. Mortazavi, J.D. Smart, Factors influencing gel-strengthening at the mucoadhesive-mucus interface, J. Pharm. Pharmacol. 46 (1994) 86–90.
[38] Y. Sudhakar, K. Kuotsu, A.K. Bandyopadhyay, Buccal bioadhesive drug delivery – a promising option for orally less efficient drugs, J. Control. Release 114 (2006) 15–40.
[39] M.E. Imam, M. Hornof, C. Valenta, G. Reznicek, A. Bernkop-Schnürch, Evidence for the interpenetration of mucoadhesive polymers into the mucous gel layer, STP Pharma. Sci. 13 (2003) 171–176.
[40] K. Park, J.R. Robinson, Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion, Int. J. Pharm. 19 (1984) 107–127.
[41] R. Riley, J. Smart, J. Tsibouklis, P. Dettmar, F. Hampson, J.A. Davis, G. Kelly, W. Wilber, An investigation of mucus/polymer rheological synergism using synthesised and characterised poly(acrylic acid)s, Int. J. Pharm. 217 (2001) 87–100.
[42] H. Park, J.R. Robinson, Mechanisms of mucoadhesion of poly(acrylic acid) hydrogels, Pharmaceut. Res. 4 (1987) 457–464.
[43] H.S. Ch'ng, H. Park, P. Kelly, J.R. Robinson, Bioadhesive polymers as platforms for oral controlled drug delivery. II: Synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers, J. Pharm. Sci. 74 (1985) 399–405.
[44] N. Peppas, Y. Huang, Nanoscale technology of mucoadhesive interactions, Adv. Drug Deliv. Rev. 56 (2004) 1675–1687.
[45] A. Bernkop-Schnürch, J. Freudl, Comparative in vitro study of different chitosan-complexing agent conjugates, Pharmazie 54 (1999) 369–371.
[46] C. Lehr, Lectin-mediated drug delivery: the second generation of bioadhesives, J. Control. Release 65 (2000) 19–29.
[47] M. Kerec, M. Bogataj, B. Mugerle, M. Gasperlin, A. Mrhar, Mucoadhesion on pig vesical mucosa: influence of polycarbophil/calcium interactions, Int. J. Pharm. 241 (2002) 135–143.
[48] J. Elliott, M. Macdonald, J. Nie, C. Bowman, Structure and swelling of poly(acrylic acid) hydrogels: effect of pH, ionic strength, and dilution on the crosslinked polymer structure, Polymer 45 (2004) 1503–1510.
[49] M.T. Nickerson, A.T. Paulson, R.A. Speers, Rheological properties of gellan solutions: effect of calcium ions and temperature on pre-gel formation, Food Hydrocolloid 17 (2003) 577–583.

[50] N. Fefelova, Z. Nurkeeva, G. Mun, V. Khutoryanskiy, Mucoadhesive interactions of amphiphilic cationic copolymers based on [2-(methacryloyloxy)ethyl]trimethylammonium chloride, Int. J. Pharm. 339 (2007) 25–32.
[51] A.K. Singla, M. Chawla, A. Singh, Potential applications of carbomer in oral mucoadhesive controlled drug delivery system: a review, Drug Dev. Ind. Pharm. 26 (2000) 913–924.
[52] V. Khutoryanskiy, Hydrogen-bonded interpolymer complexes as materials for pharmaceutical applications,, Int. J. Pharm. 334 (2007) 15–26.
[53] J. Robinson, G. Mlynek, Bioadhesive and phase-change polymers for ocular drug delivery, Adv. Drug Deliv. Rev. 16 (1995) 45–50.
[54] M. Ugwoke, E. Sam, G. VanDenMooter, N. Verbeke, R. Kinget, Nasal mucoadhesive delivery systems of the anti-parkinsonian drug, apomorphine: influence of drug-loading on in vitro and in vivo release in rabbits, Int. J. Pharm. 181 (1999) 125–138.
[55] J. Ceulemans, A. Ludwig, Optimisation of carbomer viscous eye drops: an in vitro experimental design approach using rheological techniques, Eur. J. Pharm. Biopharm. 54 (2002) 41–50.
[56] P. He, S. Davis, L. Illum, In vitro evaluation of the mucoadhesive properties of chitosan microspheres, Int. J. Pharm. 166 (1998) 75–88.
[57] S. Bautista-Baños, A.N. Hernández-Lauzardo, M.G. Velázquez-delValle, M. Hernández-López, E. AitBarka, E. Bosquez-Molina, C.L. Wilson, Chitosan as a potential natural compound to control pre and post harvest diseases of horticultural commodities, Crop Protect. 25 (2006) 108–118.
[58] P. Chassary, T. Vincent, E. Guibal, Metal anion sorption on chitosan and derivative materials: a strategy for polymer modification and optimum use, React. Funct. Polym. 60 (2004) 137–149.
[59] V. Dodane, V. Vilivalam, Pharmaceutical applications of chitosan, Pharm. Sci. Technol. 1 (1998) 246–253.
[60] A. Portero, D. Teijeiro-Osorio, M. Alonso, C. Remuñán-López, Development of chitosan sponges for buccal administration of insulin, Carbohydr. Polym. 68 (2007) 617–625.
[61] S. Rossi, F. Ferrari, M. Bonferoni, C. Caramella, Characterization of chitosan hydrochloride–mucin interaction by means of viscosimetric and turbidimetric measurements, Eur. J. Pharm. Sci. 10 (2000) 251–257.
[62] A. Bernkop-Schnürch, Mucoadhesive systems in oral drug delivery, Drug Discov. Today Tech. 2 (2005) 83–87.
[63] E.E. Hassan, J.M. Gallo, A simple rheological method for the in vitro assessment of mucin–polymer bioadhesive bond strength, Pharm. Res. 7 (1990) 491–495.
[64] A. El-Kamel, M. Sokar, V. Naggar, S. Al-Gamal, Chitosan and sodium alginate-based bioadhesive vaginal tablets, AAPS PharmSci. 4 (2002). article 44.
[65] R.J. Soane, M. Frier, A.C. Perkins, N.S. Jones, S.S. Davis, L. Illum, Evaluation of the clearance characteristics of bioadhesive systems in humans, Int. J. Pharm. 178 (1999) 55–65.
[66] I. Bravo-Osuna, C. Vauthier, A. Farabollini, G. Palmieri, G. Ponchel, Mucoadhesion mechanism of chitosan and thiolated chitosan-poly(isobutyl cyanoacrylate) core–shell nanoparticles, Biomaterials 28 (2007) 2233–2243.
[67] M. Rinaudo, Chitin, chitosan: properties and applications, Prog. Polym. Sci. 31 (2006) 603–632.
[68] A. Bernkop-Schnürch, Chitosan, its derivatives: potential excipients for peroral peptide delivery systems, Int. J. Pharm. 194 (2000) 1–13.
[69] H. Onishi, Y. Machida, Biodegradation and distribution of water-soluble chitosan in mice, Biomaterials 20 (1999) 175–182.
[70] M.A. Clark, B. Hirst, M. Jepson, Lectin-mediated mucosal delivery of drugs and microparticles, Adv. Drug Deliv. Rev. 43 (2000) 207–223.
[71] M. Wirth, K. Gerhardt, C. Wurm, F. Gabor, Lectin-mediated drug delivery: influence of mucin on cytoadhesion of plant lectins in vitro, J. Control. Release 79 (2002) 183–191.
[72] A. Bernkop-Schnürch, F. Gabor, M. Szostak, W. Lubitz, An adhesive drug delivery system based on K99-fimbriae, Eur. J. Pharm. Sci. 3 (1995) 293–299.
[73] V. Leitner, G. Walker, A. Bernkop-Schnürch, Thiolated polymers: evidence for the formation of disulphide bonds with mucus glycoproteins, Eur. J. Pharm. Biopharm. 56 (2003) 207–214.
[74] K. Albrecht, M. Greindl, C. Kremser, C. Wolf, P. Debbage, A. Bernkop-Schnürch, Comparative in vivo mucoadhesion studies of thiomer formulations using magnetic resonance imaging and fluorescence detection, J. Control. Release 115 (2006) 78–84.
[75] A. Bernkop-Schnürch, Thiomers: a new generation of mucoadhesive polymers, Adv. Drug Deliv. Rev. 57 (2005) 1569–1582.
[76] M. Roldo, M. Hornof, P. Caliceti, A. Bernkop-Schnürch, Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and in vitro evaluation, Eur. J. Pharm. Biopharm. 57 (2004) 115–121.
[77] A. Bernkop-Schnürch, A. Krauland, V. Leitner, T. Palmberger, Thiomers: potential excipients for non-invasive peptide delivery systems, Eur. J. Pharm. Biopharm. 58 (2004) 253–263.
[78] V.M. Patel, B.G. Prajapati, M.M. Patel, Formulation, evaluation, and comparison of bilayered and multilayered mucoadhesive buccal devices of propranolol hydrochloride, AAPS PharmSciTech. 8 (2007). article 22.
[79] S. Cafaggi, R. Leardi, B. Parodi, G. Caviglioli, E. Russo, G. Bignardi, Preparation and evaluation of a chitosan salt–poloxamer 407 based matrix for buccal drug delivery, J. Control. Release 102 (2005) 159–169.
[80] D. Jones, A.D. Woolfson, A. Brown, W. Coulter, C. McClelland, C. Irwin, Design, characterisation and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment of periodontal disease, J. Control. Release 67 (2000) 357–368.
[81] D. Jones, A.D. Woolfson, A. Brown, M. O'Neill, Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: In vitro release kinetics, syringeability, mechanical and mucoadhesive properties, J. Control. Release 49 (1997) 71–79.
[82] H. Junginger, J. Hoogstraate, J.C. Verhoef, Recent advances in buccal drug delivery and absorption – in vitro and in vivo studies, J. Control. Release 62 (1999) 149–159.
[83] K.K. Peh, C.F. Wong, Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties, J. Pharm. Sci. 2 (1999) 53–61.
[84] J.J. Hoogstraate, P. Wertz, P. Wertz, Drug delivery via the buccal mucosa, Pharm. Sci. Technol. 1 (1998) 309–316.
[85] N. Nafee, F. Ismail, N. Boraie, L. Mortada, Mucoadhesive buccal patches of miconazole nitrate: in vitro/in vivo performance and effect of ageing, Int. J. Pharm. 264 (2003) 1–14.
[86] M. Saettone, L. Salminen, Ocular inserts for topical delivery, Adv. Drug Deliv. Rev. 16 (1995) 95–106.
[87] J. Carlfors, K. Edsman, R. Petersson, K. Jörnving, Rheological evaluation of Gelrite® in situ gels for ophthalmic use, Eur. J. Pharm. Sci. 6 (1998) 113–119.
[88] E. Grzekowiak, Biopharmaceutical availability of sulphadicramide from ocular ointments in vitro, Eur. J. Pharm. Sci. 6 (1998) 247–253.
[89] G. Wei, H. Xu, P. Ding, S. Li, J. Zheng, Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies, J. Control. Release 83 (2002) 65–74.
[90] B. Srividya, R. Cardoza, P.D. Amin, Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system, J. Control. Release 73 (2001) 205–211.
[91] M. Hornof, W. Weyenberg, A. Ludwig, A. Bernkop-Schnürch, Mucoadhesive ocular insert based on thiolated poly(acrylic acid): development and in vivo evaluation in humans, J. Control. Release 89 (2003) 419–428.
[92] K. Vermani, S. Garg, The scope and potential of vaginal drug delivery, Pharm. Sci. Technol. 3 (2000) 359–364.
[93] Z. Pavelic, N. Skalko-Basnet, R. Schubert, Liposomal gels for vaginal drug delivery, Int. J. Pharm. 219 (2001) 139–149.
[94] C. Valenta, C. Kast, I. Harich, A. Bernkop-Schnürch, Development and in vitro evaluation of a mucoadhesive vaginal delivery system for progesterone, J. Control. Release 77 (2001) 323–332.
[95] A. Hussain, F. Ahsan, The vagina as a route for systemic drug delivery, J. Control. Release 103 (2005) 301–313.
[96] K. Barnhart, E.S. Pretorius, A. Stolpen, D. Malamud, Distribution of topical medication in the human vagina as imaged by magnetic resonance imaging, Fertil. Steril. 76 (2001) 189–195.
[97] E. Hardy, A. Jiménez, K. dePádua, L.D. Zaneveld, Women's preferences for vaginal antimicrobial contraceptives. III: Choice of a formulation, applicator, and packaging, Contraception 58 (1998) 245–249.
[98] S. Garg, R. Anderson, C. Chany, D. Waller, X. Diao, K. Vermani, L.D. Zaneveld, Properties of a new acid-buffering bioadhesive vaginal formulation (ACIDFORM), Contraception 64 (2001) 67–75.
[99] E. Amaral, A. Perdigao, M. Souza, C. Mauck, D. Waller, L. Zaneveld, A. Faundes, Vaginal safety after use of a bioadhesive, acid-buffering, microbicidal contraceptive gel (ACIDFORM) and a 2% nonoxynol-9 product, Contraception 73 (2006) 542–547.
[100] M.M. Dhondt, E. Adriaens, J. Roey, J. Remon, The evaluation of the local tolerance of vaginal formulations containing dapivirine using the Slug Mucosal Irritation test and the rabbit vaginal irritation test, Eur. J. Pharm. Biopharm. 60 (2005) 419–425.
[101] A. Darwish, E. Hafez, I. El-Gebali, S. Hassan, Evaluation of a novel vaginal bromocriptine mesylate formulation: a pilot study, Fertil. Steril. 83 (2005) 1053–1055.
[102] E. Marttin, N.M. Schipper, J.C. Verhoef, F.H.M. Merkus, Nasal mucociliary clearance as a factor in nasal drug delivery, Adv. Drug Deliv. Rev. 29 (1998) 13–38.
[103] H. Costantino, L. Illum, G. Brandt, P. Johnson, S. Quay, Intranasal delivery: physicochemical and therapeutic aspects, Int. J. Pharm. 337 (2007) 1–24.
[104] S. Pisal, A. Paradkar, K. Mahadik, S. Kadam, Pluronic gels for nasal delivery of Vitamin B12. Part I: Preformulation study, Int. J. Pharm. 270 (2004) 37–45.
[105] K. Nakamura, Y. Maitani, A. Lowman, K. Takayama, N. Peppas, T. Nagai, Uptake and release of budesonide from mucoadhesive, pH-sensitive copolymers and their application to nasal delivery, J. Control. Release 61 (1999) 329–335.
[106] L. Illum, I. Jabbal-Gill, M. Hinchcliffe, A.N. Fisher, S.S. Davis, Chitosan as a novel nasal delivery system for vaccines, Adv. Drug Deliv. Rev. 51 (2001) 81–96.
[107] C. Tas, C. Ozkan, A. Savaser, Y. Ozkan, U. Tasdemir, H. Altunay, Nasal absorption of metoclopramide from different Carbopol® 981 based formulations: in vitro, ex vivo and in vivo evaluation, Eur. J. Pharm. Biopharm. 64 (2006) 246–254.
[108] U. Bertram, R. Bodmeier, In situ gelling, bioadhesive nasal inserts for extended drug delivery: in vitro characterization of a new nasal dosage form, Eur. J. Pharm. Sci. 27 (2006) 62–71.
[109] H.O. Alpar, S. Somavarapu, K.N. Atuah, V.W. Bramwell, Biodegradable mucoadhesive particulates for nasal and pulmonary antigen and DNA delivery, Adv. Drug Deliv. Rev. 57 (2005) 411–430.
[110] A. Krauland, D. Guggi, A. Bernkop-Schnürch, Thiolated chitosan microparticles: a vehicle for nasal peptide drug delivery, Int. J. Pharm. 307 (2006) 270–277.

[111] M. Vajdy, D. O'Hagan, Microparticles for intranasal immunization, Adv. Drug Deliv. Rev. 51 (2001) 127–141.
[112] I. vanderLubben, G. Kersten, M. Fretz, C. Beuvery, J. CoosVerhoef, H. Junginger, Chitosan microparticles for mucosal vaccination against diphtheria: oral and nasal efficacy studies in mice, Vaccine 21 (2003) 1400–1408.
[113] A. Vila, A. Sánchez, C. Évora, I. Soriano, O. McCallion, M.J. Alonso, PLA-PEG particles as nasal protein carriers: the influence of the particle size, Int. J. Pharm. 292 (2005) 43–52.
[114] C.F. Kuper, J.E. Arts, V. Feron, Toxicity to nasal-associated lymphoid tissue, Toxicol. Lett. 140–141 (2003) 281–285.
[115] P. Dondeti, H. Zia, T. Needham, Bioadhesive and formulation parameters affecting nasal absorption, Int. J. Pharm. 127 (1996) 115–133.
[116] A. Streubel, J. Siepmann, R. Bodmeier, Drug delivery to the upper small intestine window using gastroretentive technologies, Curr. Opin. Pharmacol. 6 (2006) 501–508.
[117] C. Jacobs, O. Kayser, R.H. Müller, Production and characterisation of mucoadhesive nanosuspensions for the formulation of bupravaquone, Int. J. Pharm. 214 (2001) 3–7.
[118] S.S. Davis, Formulation strategies for absorption windows,, Drug Discov. Today. 10 (2005) 249–257.
[119] M. Chun, H. Sah, H. Choi, Preparation of mucoadhesive microspheres containing antimicrobial agents for eradication of *H. pylori*, Int. J. Pharm. 297 (2005) 172–179.
[120] M. Säkkinen, J. Marvola, H. Kanerva, K. Lindevall, A. Ahonen, M. Marvola, Are chitosan formulations mucoadhesive in the human small intestine? An evaluation based on gamma scintigraphy, Int. J. Pharm. 307 (2006) 285–291.
[121] A. Krauland, D. Guggi, A. Bernkop-Schnürch, Oral insulin delivery: the potential of thiolated chitosan–insulin tablets on non-diabetic rats, J. Control. Release 95 (2004) 547–555.
[122] K.P.R. Chowdary, Y.S. Rao, Mucoadhesive microspheres for controlled drug delivery, Biol. Pharm. Bull. 27 (2004) 1717–1724.
[123] M. Säkkinen, J. Marvola, H. Kanerva, K. Lindevall, M. Lipponen, T. Kekki, A. Ahonen, M. Marvola, Gamma scintigraphic evaluation of the fate of microcrystalline chitosan granules in human stomach, Eur. J. Pharm. Biopharm. 57 (2004) 133–143.
[124] D. Accili, G. Menghi, G. Bonacucina, P. Martino, G. Palmieri, Mucoadhesion dependence of pharmaceutical polymers on mucosa characteristics, Eur. J. Pharm. Sci. 22 (2004) 225–234.
[125] J.D. Smart, I.W. Kellaway, H.E. Worthington, An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery, J. Pharm. Pharmacol. 36 (1984) 295–299.
[126] C. Wong, K. Yuen, K. Peh, An in-vitro method for buccal adhesion studies: importance of instrument variables, Int. J. Pharm. 180 (1999) 47–57.
[127] S.H. Leung, J.R. Robinson, The contribution of anionic polymer structural features to mucoadhesion, J. Control. Release 5 (1988) 223–231.
[128] S.A. Mortazavi, J.D. Smart, An investigation of some factors influencing the in vitro assessment of mucoadhesion, Int. J. Pharm. 116 (1995) 223–230.
[129] M.J. Tobyn, J.R. Johnson, P.W. Dettmar, Factors affecting in vitro gastric mucoadhesion. I: Test conditions and instrumental parameters, Eur. J. Pharm. Biopharm. 41 (1995) 235–241.
[130] V. Grabovac, D. Guggi, A. Bernkop-Schnürch, Comparison of the mucoadhesive properties of various polymers, Adv. Drug Deliv. Rev. 57 (2005) 1713–1723.
[131] F. Madsen, K. Eberth, J. Smart, A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration, J. Control. Release 50 (1998) 167–178.
[132] S. Tamburic, D.M. Craig, A comparison of different in vitro methods for measuring mucoadhesive performance, Eur. J. Pharm. Biopharm. 44 (1997) 159–167.
[133] S. Rossi, M.C. Bonferoni, G. Lippoli, M. Bertoni, F. Ferrari, C. Caramella, U. Conte, Influence of mucin type on polymer–mucin rheological interactions, Biomaterials 16 (1995) 1073–1079.
[134] H.K. Batchelor, D. Banning, P.W. Dettmar, F.C. Hampson, I.G. Jolliffe, D.Q.M. Craig, An in vitro mucosal model for prediction of the bioadhesion of alginate solutions to the oesophagus, Int. J. Pharm. 238 (2002) 123–132.
[135] S. Kockisch, G. Rees, S. Young, J. Tsibouklis, J. Smart, A direct-staining method to evaluate the mucoadhesion of polymers from aqueous dispersion, J. Control. Release 77 (2001) 1–6.
[136] H. Takeuchi, J. Thongborisute, Y. Matsui, H. Sugihara, H. Yamamoto, Y. Kawashima, Novel mucoadhesion tests for polymers and polymer-coated particles to design optimal mucoadhesive drug delivery systems, Adv. Drug Deliv. Rev. 57 (2005) 1583–1594.
[137] S.S. Davis, The design, evaluation of controlled release systems for the gastrointestinal tract, J. Control. Release 2 (1985) 27–38.
[138] G. Ponchel, J. Irache, Specific and non-specific bioadhesive particulate systems for oral delivery to the gastrointestinal tract, Adv. Drug Deliv. Rev. 34 (1998) 191–219.