Multilayered hydrogel coatings covalently-linked to glass surfaces showing a potential to mimic mucosal tissues

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Multilayered hydrogel coatings can be developed on the surface of glass slides *via* layer-by-layer deposition of hydrogen-bonded interpolymer complexes formed by poly(acrylic acid) and methylcellulose. Chemical modification of the glass surface with (3-aminopropyl)triethoxysilane with subsequent layer-by-layer deposition and cross-linking of interpolymer complexes by thermal treatment allows fabrication of ultrathin hydrogel coatings, not detachable from the substrate. The thickness of these coatings is directly related to the number of deposition cycles and cross-linking conditions. An unusual dependence of the hydrogel swelling properties on the sample thickness is observed and can be interpreted by gradual transitions between two- and three-dimensional networks. The hydrogels exhibit pH-responsive swelling behaviour, achieving higher swelling degrees at pH > 6.0. These coatings can be used as model substrates to study the adhesive properties of pharmaceutical tablets and can potentially mimic the total work of adhesion observed for the detachment of mucoadhesives from porcine buccal mucosa but fail to exhibit identical detachment profiles.

Introduction

Ultrathin hydrogel films have attracted recent attention of researchers due to the numerous potential applications of these materials for fabricating functionalised and stimuli-responsive surfaces, chemical sensors, gating devices, actuators, encapsulation of cells and design of drug delivery systems.[1]

Layer-by-layer (LbL) sequential deposition of water-soluble polymers on solid substrates offers an excellent opportunity for developing ultrathin coatings with highly controlled dimensions and easily tuned properties. This approach was pioneered by Decher[2] using oppositely charged polyelectrolytes, able to form insoluble polyelectrolyte complexes due to electrostatic attraction forces. It was also successfully employed by Stockton and Rubner,[3] Wang *et al.*[4] and Sukhishvili *et al.*[5,6] for fabricating multilayered materials through the complex formation between polymers *via* hydrogen-bonding. In the past few years, a number of studies have been published on the use of LbL self-assembly *via* hydrogen-bonding for developing multilayered microcapsules, ultrathin films and coatings.[7–14] The progress in this area was summarised in two reviews published by Kharlampieva *et al.*[15,16]

Hydrogen-bonded complexes formed between poly(acrylic acid) or poly(methacrylic acid) and synthetic non-ionic polymers such as polyethylene oxide, poly(*N*-vinyl pyrrolidone), polyacrylamide, poly(*N*-isopropyl acrylamide), poly(vinyl methyl ether), poly(*N*-vinyl caprolactam) and poly(2-hydroxyethyl acrylate) are the most commonly utilised for building LbL materials.[15] We have previously demonstrated that non-ionic cellulose ethers are also able to form hydrogen-bonded interpolymer complexes (IPCs) with poly(acrylic acid) in aqueous solutions under acidic conditions. These non-ionic cellulose ethers include hydroxyethylcellulose,[17,18] methylcellulose,[19,20] hydroxypropylcellulose[20–22] and hydroxypropylmethylcellulose.[23]

The specific feature of the complexes formed by cellulose ethers is their non-stoichiometric nature, which often results in incorporation of excessive amount of poly(acrylic acid) into the IPC structure. The use of cellulose ethers for complexation opens a number of interesting opportunities for application of IPCs due to industrial importance of these polymers, their biocompatibility and non-toxicity.

To the best of our knowledge, only two studies have been reported so far on the use of non-ionic cellulose ethers for building multilayered materials by LbL approach.[7,12] We reported the formation of multilayered hydrogels using the complexation between poly(acrylic acid) (PAA) and methylcellulose (MC) *via* hydrogen-bonding.[7] These materials were prepared by LbL deposition of polymeric complexes on a glass surface. The thermal treatment of the polymeric complexes resulted in cross-linking of the polymers and the formation of films, which detached from the glass substrate upon swelling as free-standing ultrathin hydrogels. Guan *et al.*[12] used the complexation between PAA and hydroxypropylcellulose to build monodisperse hollow microcapsules by LbL assembling of IPCs onto colloidal SiO_2 particles as a template with subsequent removal of silica core by treatment with hydrofluoric acid.

Planar glass substrates, functionalised with covalently-attached hydrophilic polymeric layers were previously developed by Beyer *et al.*[24] through surface modification with (4-aminobutyl)dimethylmethoxysilane, reactive absorption of poly[(1-methylvinyl isocyanate)-alt-(maleic anhydride)] and subsequent reaction with poly(ethylene glycol) (PEG). More recently, Kim *et al.*[25] have reported the modification of

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borosilicate glass by reaction with (3-aminopropyl)-triethoxysilane and its subsequent treatment with multi-arm PEG-vinyl sulfone and dithiothreitol in a layer-by-layer fashion.

Here, we describe the possibility of preparing ultrathin hydrogel coatings that are covalently-linked to a glass surface using the layer-by-layer deposition of PAA-MC complexes with their subsequent cross-linking. These hydrogels exhibited pH-responsive swelling behaviour and were used as model substrates to study adhesive properties of pharmaceutical tablets.

Experimental

Materials

Poly(acrylic acid) (M_w 450 000), (3-aminopropyl)triethoxysilane (99%) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride were purchased from Sigma-Aldrich. Methylcellulose (Methocel® 60 HG) with 28–30% methoxyl content was supplied from Fluka (UK). The viscosity of 2 wt% methylcellulose in water at 20 °C was 35–55 mPa·s. Carbopol® 940 was received from Acros Organics (Belgium). All inorganic chemicals and organic solvents were purchased from Fisher Scientific (UK) and Sigma-Aldrich (UK). All chemicals were used without additional purification. Microscopy glass slides, 0.9–1.0 mm thick were purchased from VWR International. Deionised water was used in all experiments.

Preparation of polymer solutions and buffers

All polymer solutions were prepared by dissolving the required amount of polymer in deionised water and stirring overnight at room temperature. The pH of the polymer solutions was adjusted by adding few drops of 0.1 mol L^{-1} HCl or NaOH. The buffer solutions with desired pH were prepared by mixing 0.05 or 0.1 mol L^{-1} solutions of: (1) NaH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$; (2) $B_4Na_2O_7$, H_3BO_4 and NaOH or (3) citric acid, NaOH and HCl. Phosphate buffer saline solutions were prepared using commercial tablets purchased from Sigma-Aldrich (UK). The pH of all solutions was examined using a digital pH-meter, Metrohm 713 (Switzerland).

Multilayered film deposition

Microscopy glass slides were cleaned with Piranha solution (70 : 30 v/v % mixture of concentrated sulfuric acid and hydrogen peroxide, respectively), washed with deionised water, rinsed with methanol and dried with compressed air. Then the slides were immersed into 3 mol L^{-1} solution of 3-aminopropyltriethoxysilane in toluene and left stirring for 6.5 h. Modified glass slides were transferred into toluene, sonicated in a sonication bath for 10 min, rinsed with a new portion of toluene and then with methanol several times, dried at room temperature and kept in desiccators until required.

The glass slides modified with 3-aminopropyltriethoxysilane (APTS) were pre-coated with PAA solution to build the first deposited layer. For this purpose, 1.07 g (6.9 mM) EDC was added into 500 mL 0.2 wt % PAA solution at pH 6, stirring for 30 min. Then APTS-glass slides were immersed into solution, left stirring for 3 h, removed from solution, dried in air and kept in desiccators. Note that this layer of covalently bound PAA was not considered as the first layer in the calculation of the total number of deposited monolayers.

The multilayered coatings were developed successively by alternately immersing APTS-PAA-glass slides into 0.2 wt% aqueous polymer solutions (MC or PAA). All polymers and HCl solutions were adjusted to pH 2. Each deposition cycle involved 4 steps: dipping the glass slide into MC solution for 15 min, rinsing with HCl solution for 1 min to wash out excessive unbounded macromolecules, immersing into PAA solution for 15 min, and rinsing with HCl solution for 1 min. The process of deposition was repeated 5–25 times. The resulting multicoated glass slides were dried for at least 3 days at room temperature and kept in desiccators.

Thickness and weight of dry multilayered films

The thickness of the LbL films was measured using a Flowler IP 54 digital micrometre, and determined as a difference between the thickness of multicoated and cleaned glass slide divided by 2 (2 sides of each slide were coated in multilayer deposition procedure). The weight of the LbL coatings was calculated as the difference between the weight of a glass slide after and before layer-by-layer deposition. Both thickness and weight results are reported as a mean value from 3–5 measurements.

Cross-linking of multilayered coatings

The dry LbL coatings were cross-linked in an oven by thermal treatment at 120 °C for 2, 4 and 6 h.

Swelling and images

The thermally-treated multicoated glass slides were weighed, then immersed in buffer solutions and left for at least 2 days. Then the swollen hydrogel coatings were gently wiped with a filter paper to remove excess water and reweighed. The equilibrium swelling degrees of hydrogel coatings were calculated using the following equation:

$$\text{Swelling degree} = (w_s - w_d)/w_d \quad (1)$$

where w_s and w_d are the weights of swollen and dry multicoated glass slide, respectively. The measurement of the swelling degree of the hydrogel coatings by the above method was possible because the weight of glass slides remains unchanged during the swelling/drying experiments. It should also be noted that the gentle and ultrathin nature of hydrogel coatings resulted in certain technical difficulties to measure their swelling degrees, which may have caused considerable variability between the individual measurements and some data points to be out of general trends.

Images of LbL hydrogel coatings were recorded with Leica EZ4D stereomicroscope.

X-ray photoelectron spectroscopy (XPS)

XPS measurements of dry samples were performed on a Kratos AXIS Ultra-DCD photoelectron spectrometer with a monochromatic Al Kα X-ray source ($h\nu$ = 1486.6 eV) at a power of 150 W and 20 eV of a pass energy. Survey scans were run at

160 eV pass energy and spectra obtained at 90° take off angle. All analysis was done using CASA XPS and spectra were calibrated to 285 eV for the most intense C (*1s*) peak.

Mucoadhesive tablets preparation and characterisation

Model mucoadhesive tablets were prepared by the direct compression of powder mixtures. Carbopol® 940 and 2.5 w/w % of magnesium stearate (as a lubricant) were mixed together in a Willy A. Bachofen AG Maschinenfabrik mixer (Switzerland), and then compressed using a Riva SA Minipress MII single punch tablet press (Argentina). The tablets had the following characteristics: 44.2 ± 1.1 mg in weight, diameter of 6.0 ± 0.0 mm, thickness of 2.8 ± 0.1 mm and hardness of 29.0 ± 3.6 N. The hardness of the tablets was assessed using a 6D Tablet tester, Copley Scientific. Dimensions of tablets were measured with a digital micrometre, Flowler IP 54.

Adhesion of tablets to hydrogel coatings

The adhesive properties of tablets towards multilayered PAA-MC hydrogels, porcine buccal mucosa and other substrates were evaluated using a TA.XT plus Texture Analyser (Stable Micro System, Surrey, UK). Porcine buccal mucosal tissues taken from female Great White pigs weighing 65–75 kg were obtained from MutchMeats Ltd (UK). These tissues were collected immediately after the slaughter of the animals and were stored frozen at −20 °C. Before testing, the mucosal tissues were defrosted in water at 35–37 °C. The swollen hydrogel-coated glass slide was clamped in a circle shape holder, immersed in solution of phosphate buffered saline (pH 7.0) covering 1–2 mm hydrogel surface and equilibrated at 37 ± 1 °C for 20 min. Carbopol tablets were attached to a mobile probe (cylindrical, P/6) with a double side adhesive tape. The probe was lowered at a speed of 1 mm s^{-1} until it reached the hydrogel surface. The contact time 1 min and contact force 0.1 N were applied. Then the probe was withdrawn at a rate of 0.05 mm s^{-1} until the complete detachment of tablet from hydrogel surface was observed. The maximum force of detachment and work of adhesion (the area under the force/distance curve) were determined directly using Texture Exponent 32 software package. All measurements were performed at least 5–6 times and adhesion parameters (force of detachment and total work of adhesion) were calculated as a mean ± standard deviation.

Results and discussion

Fabrication of hydrogel multilayers

In order to fabricate non-detachable multilayered PAA-MC films, the surface of microscopy glass slides was activated by treatment with 3-aminopropyltriethoxysilane (APTS). This treatment results in formation of amino-functionalised glass, able to attract negatively-charged macromolecules of PAA electrostatically. The deposition of PAA on amino-functionalised glass was undertaken in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride to facilitate the formation of covalent (amide) bonds between carboxylic groups of PAA and amino-groups on the glass surface. After the first PAA deposition was completed, it was followed by alternate

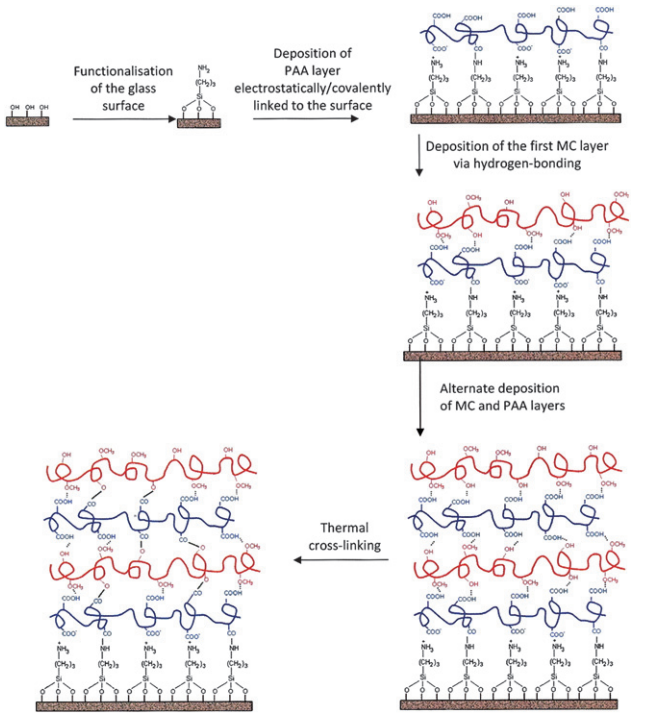

Fig. 1 Scheme of glass slide modification through amino-functionalisation, LbL deposition and cross-linking.

deposition of MC and PAA as described in our previous paper.[7] The resulting coatings were dried and cross-linked by thermal treatment in air at 120 °C for 2, 4 and 6 h (Fig. 1).

X-ray photoelectron spectroscopy (XPS) was used to follow the amino-functionalisation of glass slides and subsequent deposition of polymers (Fig. 2). The XPS spectrum of unmodified glass slide shows the traces of nitrogen (signal N 1s), which is in agreement with the data reported by Harbers *et al.*[26] The spectrum of a microscopy slide modified by treatment with APTS after its subsequent purification confirms a significant increase in a nitrogen content, which is related to the covalent attachment of amino-group moieties to the glass surface. The amino-modified glass coated with polymeric layers shows a gradual

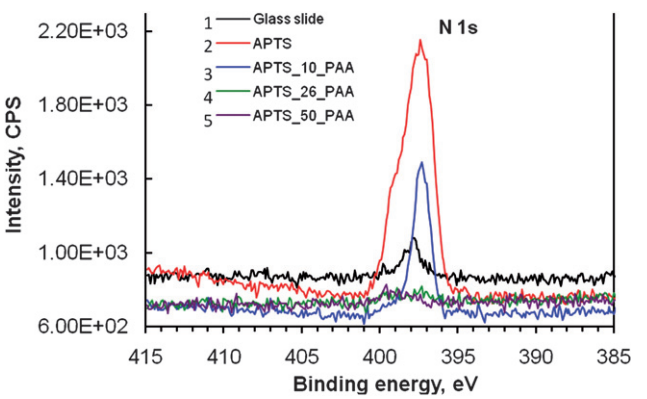

Fig. 2 XPS-spectra of an unmodified glass slide (1), amino-functionalised glass (2), amino-functionalised glass with 10 (3), 26 (4) and 50 (5) deposited monolayers. PAA monolayer was always a top layer in all LbL samples.

disappearance of the N 1s signal: the presence of 10 monolayers of PAA and MC halves the intensity of this signal, which finally disappears when the number of layers reaches 26. The disappearance of the N 1s signal is caused by the screening of the nitrogen-rich layer with nitrogen-free polymer coating as it becomes thicker.

The process of multilayer deposition was also followed by measuring the thickness of dry coatings with a digital micrometre and estimation of the relative gain in a sample weight per 1 g of glass (Fig. 3). A linear growth in the coating relative weight with the number of deposited monolayers can be observed ($R^2 = 0.94$), whereas the dependence of the coating thickness better fits with a 3rd order polynomial function. Overall, both the weight gain and the coating thickness can be easily controlled by the number of deposited cycles.

The cross-linked coatings, once immersed in phosphate buffer saline solution (pH 7.0), begin to swell and form hydrogel layers not detachable from the surface of the slides. The evaluation of these swollen coatings using optical microscopy reveals that the thickness of hydrogels correlates well with the number of deposited monolayers (Fig. 4). For example, the thicknesses of multilayered hydrogels consisting of 20, 40 and 50 monolayers were found to be 254 ± 52, 377 ± 17 and 623 ± 32 μm, respectively. Thus, depending on the number of the monolayers in the coating there is approximately a 50–100-fold increase in the coating thickness upon its swelling.

The degrees of multilayered hydrogels swelling in phosphate buffer saline (pH 7.0), determined gravimetrically, reveal a very interesting trend (Fig. 5). When the number of monolayers in the samples changes from 10 to 30, the degrees of swelling have a tendency to increase, whereas the swelling of hydrogels consisting of larger numbers of layers show less pronounced dependence on the sample thickness. For conventional hydrogels (macro-hydrogels), the degree of swelling is usually independent on the sample dimensions but depends mainly on the chemical nature of a hydrophilic polymer and its cross-linking density. The unusual dependence of the swelling properties of ultrathin hydrogels on the sample thickness is likely to be related to the predominant absorption of water by the sample surface rather than its bulk property. In this case, the ultrathin hydrogel behaves like a two-dimensional material whose water-sorption capacity is relatively low. The possibility of designing two-dimensional polymeric materials by LbL technique was recently predicted by Sakamoto *et al.*,[27] emphasising that the growth in the number of layers can result in a gradual transition from a two-dimensional to a three-dimensional material. Here we demonstrated this transition, which clearly affects the swelling properties of the hydrogel films. Indeed, when the number of PAA-MC hydrogel layers is greater than 30, the growth in the swelling degree starts to level off. It is possibly related to a gradual acquisition of a three-dimensional structure, whose capability to absorb water does not depend on sample dimensions. Additionally, when the hydrogels behave like two-dimensional materials (*i.e.* number of layers is less than 20), the degree of their swelling is not significantly affected by the cross-linking density, determined by the time of thermal treatment.

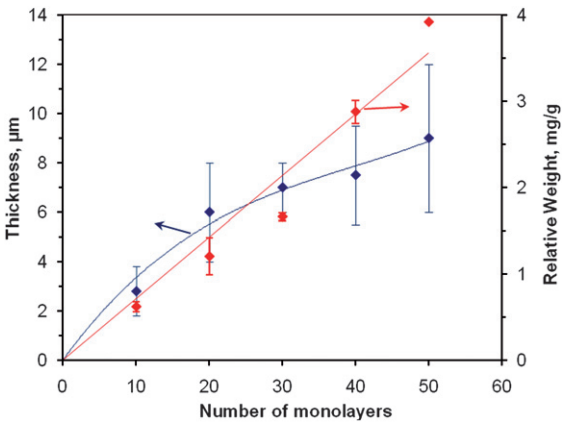

Fig. 3 Dependence of the coating thickness and relative weight on the number of deposited monolayers.

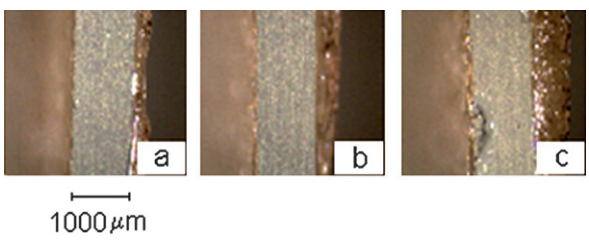

Fig. 4 Microphotographs of hydrogel coatings composed of 20 (a), 40 (b) and 50 multilayers (c), swollen in phosphate buffer saline (pH 7.0).

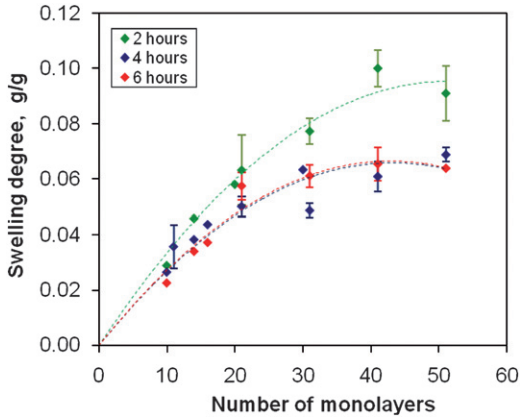

Fig. 5 Dependence of the equilibrium swelling degree of the cross-linked coatings in phosphate buffer saline (pH 7.0) on the number of deposited monolayers. Thermal treatment time: 2, 4 and 6 h at 120 °C.

As the number of layers increases, the effect of thermal treatment time on the swelling properties becomes more pronounced. The 30–50-layered samples treated for 2 h show notably higher swelling capacity because of their lower cross-linking density compared to the samples treated for 4 and 6 h. No significant difference is observed between the swelling capacities of the samples treated for 4 and 6 h.

The multilayered ultrathin hydrogels also exhibit pH-responsive swelling behaviour (Fig. 6). Under acidic conditions (pH < 4), the samples remain relatively hydrophobic and do not absorb water. This is related to the formation of hydrogen-bonded interpolymer complexes between PAA and MC, which results in reduced affinity of their functional groups to water. The

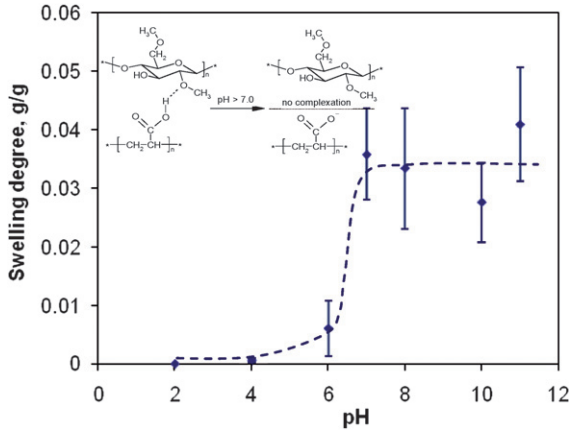

Fig. 6 Equilibrium swelling degree of 10-layered hydrogel-coatings as a function of pH. Thermal treatment: 4 h at 120 °C. Inset: Scheme of dissociation of hydrogen bonds (decomplexation) between PAA and MC.

materials display a sharp increase in their swelling capacity at pH 6–7 due to the dissociation of hydrogen bonds and ionisation of carboxylic groups.

At pH > 7, the swelling degree of the hydrogels remains pH-independent as all carboxylic groups are fully ionised (Fig. 6, inset) and the material reached its maximal water-sorption capacity.

Adhesion of tablets

The ability of certain hydrophilic polymers to adhere to mucosal membranes, referred as mucoadhesion, has been widely used for developing drug delivery systems for ocular, nasal, buccal, oral, vaginal and rectal administration.[28–30] Mucoadhesion is a complex phenomenon whose mechanisms are still not well understood. The theories that are most commonly discussed in conjunction with mucoadhesion are the electronic, absorption, diffusion and wetting theories.[31,32] The *electronic theory* assumes that transfer of electrons occurs between the mucus and the mucoadhesive due to differences in their electronic structures. This electron transfer leads to the formation of a double electric layer at the interface and results in attraction between the dosage form and the substrate. The *absorption theory* concerns attraction between the mucus and the mucoadhesive achieved *via* molecular bonding caused by secondary forces such as hydrogen bonding and van der Waals forces. The *diffusion theory* considers interpenetration and physical entanglement of the mucus protein and polymer chains of the mucoadhesive. The *wetting theory* correlates the surface tension of the mucus and the mucoadhesive with the ability of the mucoadhesive to swell and spread on the mucus layer. In isolation, none of these theories can explain mucoadhesion by the many and varied pharmaceutical formulations that have been developed. Indeed, mucoadhesion probably results from combinations of these four mechanisms.

Mucosal tissues harvested from animals are commonly used to assess the mucoadhesive potential of polymers by studying the detachment of dosage forms. Although the majority of mucoadhesive studies reported the use of animal tissues obtained from slaughterhouses, numerous publications can be found where biological tissues were taken directly after sacrificing laboratory-bred animals (see ref. 33–35 as examples). Moreover, the results obtained from these studies often give poor reproducibility due to variable properties of biological substrates. Clearly the development of an alternative testing methodology, which does not involve animal experiments in assessment of mucoadhesives, is of significant importance. Wet glass surface was previously evaluated by Shojaei *et al.*[36] as a potential substrate to test mucoadhesive properties of tablets and compared with porcine buccal mucosa. However, it was demonstrated that the forces of adhesion obtained on the wet glass surface showed no correlation with those obtained in contact with porcine buccal mucosa, largely since the glass surface is essentially flat and hard meaning the initial contact is dissimilar to that with soft tissue. In fact, the forces of adhesion generated on the glass surface were an order of magnitude different than those from contact with porcine buccal mucosa. A more successful attempt was reported by Choi and co-workers,[37] who studied the adhesion of compositions based on poly(acrylic acid) - non-ionic polymer complexes to a polypropylene plate. They claimed that there was a relatively good correlation between the adhesive force of the polymeric dosage form to pig intestinal mucosa and that of the dosage form to the polypropylene plate. However, the use of a plastic material as a substrate mimicking the natural mucosal tissue is questionable because of the significant differences in their mechanical properties, chemical composition and physicochemical characteristics.

In the present work, we tested the possibility of using glass surfaces coated with non-detachable ultrathin hydrogel layers as potential substrates for evaluating mucoadhesive properties of model dosage forms. It was believed that the soft, wet and porous structure of hydrogels should mimic the adhesive properties of mucosal tissues better, compared to unmodified glass or plastics. Model dosage forms were prepared based on Carbopol® 940, a commercial weakly-cross-linked derivative of poly(acrylic acid), which is a typically used mucoadhesive in numerous pharmaceutical formulations.

Fig. 7 shows typical profiles recorded for detachment of Carbopol® 940 tablets from porcine buccal mucus, a glass slide,

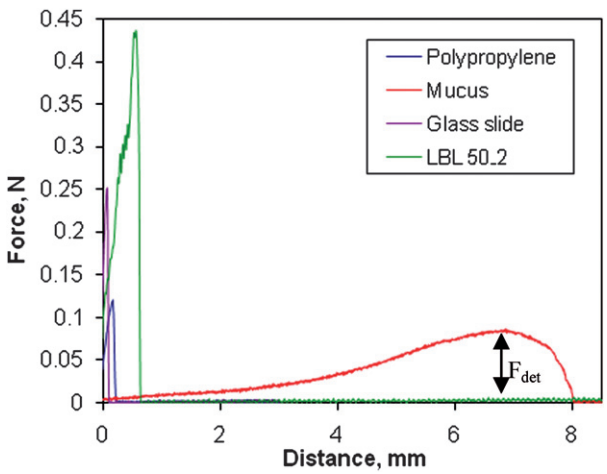

Fig. 7 Typical curves recorded for detachment of Carbopol® 940 tablets from polypropylene plate, glass slide, mucus and best performing 50-monolayered hydrogel, cross-linked by thermal treatment for 2 h.

a polypropylene plate and a glass slide coated with a multilayered hydrogel. It should be noted that the two main parameters such as the force of detachment and the total work of adhesion can be determined from these profiles. The force of detachment is defined as a maximum force required detaching a tablet from a surface and it is a maximum on each detachment curve (this force F_{det} is shown by an arrow on the profile of tablet detachment from the porcine mucus). The total work of adhesion is defined as the area under the detachment curve. These parameters have often been reported in research literature on mucoadhesives.[28] However, because of the limited availability of the instruments suitable for automatic measurements of the detachment process and data processing, many researchers have used the force of detachment as the only parameter to characterise the mucoadhesive performance of dosage forms.[36–38]

This parameter can only give limited information on the adhesion between two flat, non-deformable surfaces without interpenetration between the components of these materials. A more reliable characterisation of mucoadhesive properties can be achieved by analyzing the force of detachment and total work of adhesion in combination,[28] or by looking at the detachment profiles. Indeed, the detachment of mucoadhesive tablets from a polypropylene plate gives F_{det} values similar to those recorded with the porcine buccal mucosa, which agrees with the report of Choi *et al.*[37] However, the total work of adhesion as well as the detachment profiles are significantly different, which compromises the possibility of using polypropylene as a mucosa mimic. The force of detachment of mucoadhesive tablets from unmodified glass surface is notably higher compared to the data obtained for porcine mucus, which is also in good agreement with the report of Shajaei *et al.*[36] The lack of elasticity of both polypropylene and glass as well as the impossibility of interpenetration between the components of the mucoadhesive dosage form and a substrate make the detachment profiles very sharp and narrow. When the glass surface is modified with an elastic hydrogel layer, the force required to detach a tablet from the substrate is much greater compared to the unmodified slide. This is possibly related to the facilitated diffusion and interpenetration between the macromolecules of a dosage form and a substrate network. The detachment profiles in this case become more extended and, in combination, these will result in the work of adhesion values approaching the ones observed for the natural mucosa.

Fig. 8 summarises the data on the total work of adhesion recorded for detachment of Carbopol® 940 tablets from porcine buccal mucosa, polypropylene, glass and a series of glass samples coated with hydrogel layers of varied thickness and cross-linking degree.

The total work of adhesion recorded for detaching a tablet from unmodified glass and polypropylene is markedly lower compared to the natural mucosa. However, when the glass surface is coated with a hydrogel layer, the total work of adhesion increases and becomes higher for samples having greater number of multilayers. The samples of hydrogels which were cross-linked by thermal treatment for 2 h exhibit better mucosa-mimetic ability compared to the coatings of greater cross-linking density (*i.e.* lower hydration/swelling degree). For the samples cross-linked for 6 h, the total work of adhesion increases with a number of deposited layers insignificantly, which is likely related to their lower hydration/swelling degrees.

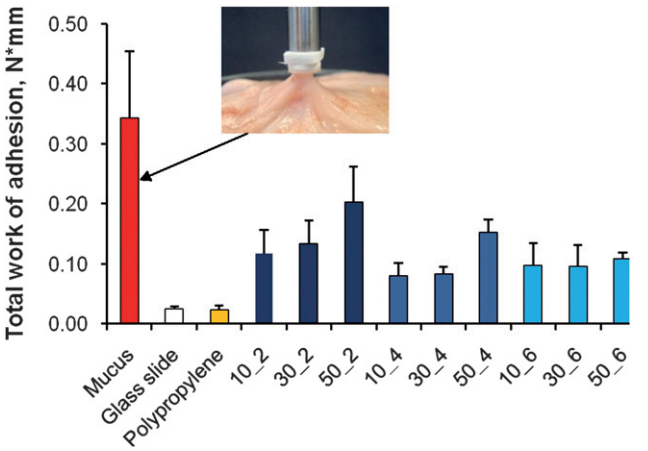

Fig. 8 Total work of adhesion of Carbopol® 940 tablets to porcine buccal mucus, glass slide, polypropylene plate and glass slides with hydrogel coatings. The first number in a sample code shows the number of deposited monolayers, the second number is the time of the material thermal treatment in hours (for example, 50_2 means that 50-monolayers were deposited and were cross-linked by thermal treatment for 2 h). Each detachment experiment was performed with at least 4–5 samples and the results are presented as a mean ± standard deviation. Inset: Image showing a detachment of Carbopol® 940 tablet from buccal mucosa.

We can conclude that neither force of detachment, nor total work of adhesion, nor even the use of these parameters in combination can give a complete description of mucoadhesive properties of a dosage form. Very simple plastic materials such as polypropylene plates can sometimes show mucosa-mimetic properties if only detachment forces are considered. The ultrathin hydrogel coatings developed in this work have shown some potential to mimic the total work of adhesion, but failed to exhibit similarity with porcine mucosa in terms of their detachment profiles. We believe that the detachment profiles for a given dosage form/substrate can be considered as a unique "signature" of mucosal adhesion and provides a complete description of the mucoadhesive performance. Our results allow concluding that further improvement in mucosa-mimetic characteristics can possibly be achieved by using thicker hydrogel samples with optimised swelling characteristics, levels of hydration, elasticity and porosity.

Conclusions

In this work we have demonstrated a successful design of ultrathin hydrogel coatings covalently bound to a glass surface. This was achieved through the modification of the glass surface by treating it with (3-aminopropyl)triethoxysilane with subsequent layer-by-layer deposition of interpolymer complexes formed by poly(acrylic acid) and methylcellulose. The thermal treatment of dry coatings at 120 °C allows cross-linking of polymers which, after exposure to an aqueous environment, form non-detachable, ultrathin hydrogels. The thickness of the hydrogel coatings is highly dependent on the number of deposited layers and the degree of swelling of these materials can easily be tuned by varying the cross-linking conditions (thermal treatment time).

The swelling properties of the hydrogels were found to be pH dependent with a minimal water-uptake at pH < 6 and their

dramatic increase at higher pHs. The swelling properties of these hydrogels have also showed unusual dependence on the sample thickness, which is related to a gradual transition from a two- to a three-dimensional network structure.

The hydrogel coatings were used as model substrates to study mucoadhesive properties of tablets in comparison with porcine buccal mucosa. Although we did not achieve a material with perfect mucosa-mimetic characteristics, these experiments provided useful information for understanding adhesion phenomena and for further development of mucosa-mimicking materials.

Acknowledgements

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References

1 I. Tokarev and S. Minko, *Soft Matter*, 2009, **5**, 511.
2 G. Decher, *Science*, 1997, **277**, 1232.
3 W. B. Stockton and M. F. Rubner, *Macromolecules*, 1997, **30**, 2717.
4 L. Wang, Z. Wang, X. Zhang, J. Shen, L. Chi and H. Fuchs, *Macromol. Rapid Commun.*, 1997, **18**, 509.
5 S. A. Sukhishvili and S. Granick, *J. Am. Chem. Soc.*, 2000, **122**, 9550.
6 S. A. Sukhishvili and S. Granick, *Macromolecules*, 2002, **35**, 301.
7 O. V. Khutoryanskaya, A. C. Williams and V. V. Khutoryanskiy, *Macromolecules*, 2007, **40**, 7707.
8 D. E. Zhunuspayev, G. A. Mun, P. Hole and V. V. Khutoryanskiy, *Langmuir*, 2008, **24**, 13742.
9 J. Seo, J. L. Lutkenhaus, J. Kim, P. T. Hammond and K. Char, *Langmuir*, 2008, **24**, 7995.
10 I. Erel-Unal and S. A. Sukhishvili, *Macromolecules*, 2008, **41**, 8737.
11 A. N. Zelikin, Q. Li and F. Caruso, *Chem. Mater.*, 2008, **20**, 2655.
12 Y. Guan, Y. Zhang, T. Zhou and S. Zhou, *Soft Matter*, 2009, **5**, 842.
13 X. Liu, B. Dai, L. Zhou and J. Sun, *J. Mater. Chem.*, 2009, **19**, 497.
14 W. Lin, Y. Guan, Y. Zhang, J. Xu and X. X. Zhu, *Soft Matter*, 2009, **5**, 860.
15 E. Kharlampieva and S. A. Sukhishvili, *J. Macromol. Sci. Part C: Polym. Rev.*, 2006, **46**, 377–395.
16 E. Kharlampieva, V. Kozlovskaya and S. A. Sukhishvili, *Adv. Mater.*, 2009, **21**, 3053.
17 Y. J. Bo, V. V. Khutoryanskiy, G. A. Mun and Z. S. Nurkeeva, *Polym. Sci. Ser. A*, 2002, **44**, 1094.
18 G. A. Mun, Z. S. Nurkeeva, V. V. Khutoryanskiy and A. V. Dubolazov, *Polym. Sci. Ser. B*, 2003, **45**, 361.
19 Z. S. Nurkeeva, G. A. Mun, V. V. Khutoryanskiy and R. A. Mangazbaeva, *Polym. Int.*, 2000, **49**, 867.
20 V. V. Khutoryanskiy, A. V. Dubolazov, Z. S. Nurkeeva and G. A. Mun, *Polym. Sci. Ser. B*, 2003, **45**, 89.
21 V. V. Khutoryanskiy, M. G. Cascone, L. Lazzeri, N. Barbani, Z. S. Nurkeeva, G. A. Mun and A. V. Dubolazov, *Polym. Int.*, 2004, **53**, 307.
22 A. V. Dubolazov, Z. S. Nurkeeva, G. A. Mun and V. V. Khutoryanskiy, *Biomacromolecules*, 2006, **7**, 1637.
23 R. A. Mangazbaeva, G. A. Mun, Z. S. Nurkeeva and V. V. Khutoryanskiy, *Polym. Int.*, 2006, **55**, 668.
24 D. Beyer, T. M. Bohanon, W. Knoll, H. Ringsdorf, G. Elender and E. Sackmann, *Langmuir*, 1996, **12**, 2514.
25 J. Kim, B. K. Wacker and D. L. Elbert, *Biomacromolecules*, 2007, **8**, 3682.
26 G. M. Harbers, K. Emoto, C. Greef, S. W. Metzger, H. N. Woodward, J. J. Mascali, D. W. Grainger and M. J. Lochhead, *Chem. Mater.*, 2007, **19**, 4405.
27 J. Sakamoto, J. van Heijst, O. Lukin and A. D. Schluter, *Angew. Chem., Int. Ed.*, 2009, **48**, 1030.
28 V. Grabovac, D. Guggi and A. Bernkop-Schnurch, *Adv. Drug Delivery Rev.*, 2005, **57**, 1713.
29 K. H. Edsman and H. Hägerström, *J. Pharm. Pharmacol.*, 2005, **57**, 3.
30 G. P. Andrews, T. P. Laverty and D. S. Jones, *Eur. J. Pharm. Biopharm.*, 2009, **71**, 505.
31 D. Dodou, P. Breedveld and P. A. Wieringa, *Eur. J. Pharm. Biopharm.*, 2005, **60**, 1.
32 J. Smart, *Adv. Drug Delivery Rev.*, 2005, **57**, 1556.
33 A. D. Ascentiis, J. L. deGrazia, C. N. Bowman, P. Colombo and N. A. Peppas, *J. Controlled Release*, 1995, **33**, 197.
34 L. S. Nielsen, L. Schubert and J. Hansen, *Eur. J. Pharm. Sci.*, 1998, **6**, 231.
35 L. Bromberg, M. Temchenko, V. Alakhov and T. A. Hatton, *Int. J. Pharm.*, 2004, **282**, 45.
36 A. H. Shojaei, J. Paulson and S. Honary, *J. Controlled Release*, 2000, **67**, 223.
37 H.-K. Choi, O.-J. Kim, C.-K. Chung and C.-S. Cho, *J. Appl. Polym. Sci.*, 1999, **73**, 2749.
38 J. Varshosaz and Z. Dehghan, *Eur. J. Pharm. Biopharm.*, 2002, **54**, 135.