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# The use of simple dynamic mucosal models and confocal microscopy for the evaluation of lyophilised nasal formulations

Fiona McInnes, Alan J. Baillie and Howard N. E. Stevens

## Abstract

A range of methods is reported in the literature for assessing hydration and adhesion parameters in the performance of nasal bioadhesive formulations; however, these tests do not always represent the dynamic conditions in the nasal cavity. Lyophilised formulations intended for nasal administration were evaluated using in-vitro tests designed in an attempt to mimic relevant processes in the nasal cavity, and intended to discriminate between different formulations. Initial investigative studies using scanning electron microscopy revealed that the lyophilisate had a highly porous internal structure, expected to provide an ideal porous pathway for re-hydration. Vapour sorption analysis demonstrated substantial weight gain of the lyophilisates on exposure to 95% relative humidity, ranging from 38% to 66%. Agar was used as a synthetic mucosal model designed to provide a standardised quantity of water available for rehydration of the formulations in in-vitro tests. A dynamic adhesion test and a texture analyser sliding test were designed to quantify different aspects of the spreading and adhesion of the hydrating formulations on the synthetic mucosal surface. Examination of the lyophilised formulations using confocal microscopy allowed visualisation and quantification of the initial rate of water ingress into the lyophilisates, which was found to consist of an initial rapid phase, followed by a slower steady-state phase. The results demonstrated that the use of a combination of methods representing the dynamic conditions of the nasal cavity is advisable in order to evaluate a formulation fully and to avoid misleading conclusions.

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, UK

Fiona McInnes, Howard N. E. Stevens

Evotec (Scotland) Ltd, West of Scotland Science Park, Glasgow G20 0XA, UK

Alan J. Baillie

**Correspondence:** Fiona McInnes, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, The John Arbuthnott Building, 27 Taylor Street, Glasgow G4 0NR, UK. E-mail: fiona.mcinnes@strath.ac.uk

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## Introduction

Nasal drug delivery is a widely investigated alternative to oral drug delivery for many researchers. The nasal mucosa offers the opportunity for direct absorption into the arterial circulation, avoiding first-pass hepatic clearance and the harsh conditions of the gastrointestinal tract. This route may allow use of smaller doses than with oral delivery, with fewer side-effects. Nasal drug delivery may also prove to be an acceptable route of administration for patients for compounds that cannot be administered orally. One of the major drawbacks of administration via conventional nasal spray dosing is the rapid mucociliary clearance of the nasal mucosa, with a clearance half-life of about 15 min (Mygind & Dahl 1998), which results in a relatively short window for absorption of the therapeutic compound. In order to overcome this problem, many researchers have reported the use of bioadhesive materials, particularly polymers, in nasal drug delivery.

The main purpose of a bioadhesive is to bind in some way with the mucous layer that covers the nasal epithelium, thereby decreasing its rate of clearance from the nasal cavity. This increased contact time with the mucosa extends the opportunity for absorption (Dondeti et al 1996). This approach also provides the potential to adapt the formulation for controlled release of the drug if desired (Alur et al 1999). In combination with factors such as molecular weight, crosslinking, concentration and pH (Ugwoke et al 2001), the degree of swelling or water absorption exhibited by a polymer is considered to be an important factor in bioadhesive behaviour (Dondeti et al 1996; Nakamura et al 1996; 1999), as the polymer must have the ability to take up water from the mucosal surface (Illum et al 1987).

Water uptake and swelling increase the extent of bioadhesion by forming a gel structure with increased flexibility of the polymer chains (Alur et al 1999), permitting intimate contact between the polymer and the mucosal surface by allowing the chain sections of the polymer to interact and become entangled with the glycoproteins of the mucus (Illum et al 1987; Nakamura et al 1999). This entanglement appears to have an important role in bioadhesion (Lee et al 2000), altering the properties of the mucous layer and reducing the rate of mucociliary clearance of the formulation.

It is also thought that an important contributor to the bioadhesive effect is the spreading of the hydrated polymer over the mucosa (Peppas and Buri 1985), which in some way alters the nature of the mucous layer, reducing clearance. Although it is generally expected that a polymer which swells rapidly will adhere to the mucous layer more readily (Dondeti et al 1996), conversely it has also been reported that overhydration can occur, with a resultant loss of adhesion (Henriksen et al 1996, Smart et al 1984).

Researchers describe various methods used in attempts to compare formulations, ranging from a flow of material over excised mucosal tissue (Batchelor et al 2002) to mechanical measurement of the force of adhesion, such as tensile testing (Mortazavi & Smart 1995) and the use of the texture analyser (TA) (Eouani et al 2001, Jones et al 1997). In these tests, the force required to remove bioadhesive formulations from a substrate is used to quantify adhesive properties. The substrate employed can vary, ranging from an inert glass surface (Maggi et al 1994) to ex-vivo mucosal substrates such as bovine buccal mucosa (Park & Munday 2002, Maggi et al 1994), although the use of excised tissue can introduce a degree of variability, as no two pieces of tissue will be entirely similar. Potential drawbacks of measuring adhesion using a method where the substrate is withdrawn from a surface at a 90° angle include the lack of identification of exactly which section of the adhesive structure is being measured – the fracture of the adhesive gel itself or the actual adhesive interface – and that perpendicular forces are not representative of the forces that act on the formulation in-vivo. When a formulation is administered to the nasal cavity and adheres to the mucosa, the force acting on it will be the action of the cilia beating, moving the foreign object towards the posterior of the nose, in an attempt to remove it. Thus, the force acting on the nasal dose is that of a sliding action, parallel to the mucosal surface.

A previous report has described the use of a lyophilised nasal insert formulation to achieve prolonged nasal absorption of nicotine in sheep (McInnes et al 2005). As hydration (contact/wetting stage) and spreading (consolidation stage) of the formulation to allow polymer chains to interact with glycoproteins in the mucous layer will be essential steps in the adhesion process (Smart 2005), a series of tests was devised to investigate these properties. Hydration was assessed using conventional water-uptake measurements, and rate of water ingress was quantified using confocal microscopy. Adhesion tests were designed to give particular emphasis to mimicking the dynamic process of simultaneous hydration, sliding and spreading within the nasal cavity, using agar as a standardised synthetic mucosal surface with water available for hydration.

## Materials and Methods

### Materials

K4MP and K100LV hydroxypropylmethylcellulose (HPMC) powders were gifts from Dow Chemicals (Michigan, IL, USA). D(−)Mannitol powder (general purpose reagent), polyvinylpyrrolidone (molecular weight 70000; PVP), ultra-pure agar and glass sintered discs (diameter 20 mm) were all purchased from BDH (VWR, Poole, UK). Vacuum-formed blister packaging cavities with 12 mm diameter wells were a gift from Quintiles (Livingston, UK) and were used as supplied. Polythene microcentrifuge tubes (0.2 mL) were obtained from Life Sciences (Basingstoke, UK).

### Methods

*Preparation of lyophilised formulations*

Gels containing different concentrations of HPMC (formulations A–F) were prepared as described in Table 1. Mannitol was included in some formulations to ensure mechanical strength of the lyophilisates, and the effect of removing this excipient was also studied. The addition of PVP to the formulation in an attempt to enhance adhesive effect was also studied in some cases. HPMC gels were lyophilised using a Virtis Advantage Freeze Drier (Virtis, New York, USA) with preset cycle stages: freezing (reducing temperature from −30°C to −60°C in 10° increments over 8 h,), primary drying (increasing temperature from 10°C to 22°C in 5° increments, with chamber pressure decreasing from 100 to 40 millitorre, over 18 h) and post heat (10 min at 25°C, 40 millitorre). All lyophilised formulations were transferred to a desiccator at room temperature immediately following completion of freeze drying, and samples were removed immediately before experimentation.

*Scanning electron microscopy (SEM)*

Samples were prepared by gently prising apart a section of the lyophilisate, in such a way as to expose an internal area without destroying the structure. The sample was fixed in place by means of copper electrical tape, and gold coated using a Polaron SC515 SEM Coating System (Bio-Rad sputter coater, Bio-Rad Ltd, Hemel Hempstead, UK). SEM

**Table 1** Composition (% w/w) of hydroxypropylmethylcellulose gels before lyophilisation

| Formulation | K4MP | K100LV | Mannitol | PVP |
|---|---|---|---|---|
| A | 1% | – | 1% | – |
| B | 2% | – | 1% | – |
| C | 3% | – | 1% | – |
| D | 2% | – | – | – |
| E | – | 2% | 1% | – |
| F | 2% | – | – | 1% |

images were obtained using a Phillips SEM 500 (Guildford, UK), with a spot size of 320 Å and 12 KV intensity.

*Dynamic vapour sorption (DVS)*

Samples of 10–20 mg were subjected to a controlled cycle of changing relative humidity (RH), beginning with an initial drying phase at 0% RH, after which RH was increased in stepwise increments to 95% RH. RH was decreased through the same steps, and these increasing and decreasing RH cycles were repeated. Progression to the next increment of a cycle was triggered when either the weight of the sample had remained constant, such that the rate of weight change over 20 min was less than 0.002 mg $min^{-1}$, or that the maximum step time of 999 min had been reached. Changes in the weight of the sample are expressed as a percentage of its dry weight.

*Confocal scanning laser microscopy (CSLM)*

Disc-shaped lyophilates were prepared as described for the dynamic adhesion test, with the exception that 0.6 g $L^{-1}$ sodium fluoroscein was added to the gel before lyophilisation. Lyophilised discs were compressed in a device designed to ensure consistent depth of sample for microscopy, following which a cover slip was added and the sample was analysed in the device. The device enabled the compartment containing the sample to be flooded with a weak water/rhodamine mixture during microscope scanning, ensuring that the disc was completely surrounded with solution available for rehydration. Radial ingress of water into the sample was analysed using a Bio-Rad MRC 1024ES laser confocal scanning imaging system (Bio-Rad Ltd, Hemel Hempstead, UK) coupled to a Nikon upright microscope using the argon laser line at 488 nm. Scans were performed using $10 \times 0.25$NA objective, at 1 or 2 s intervals over an area $1400\,\mu m \times 1400\,\mu m$. Scans were performed during initial hydration at the edge of the lyophilisate, and at a point closer to the centre of the disc when an apparent steady rate of hydration had been reached.

*Simple hydration test*

A simple hydration test was carried out to assess the extent of water uptake by nasal inserts from the agar surface during experimental procedures. Nasal inserts were weighed and placed on an agar surface (Ultrapure agar, 12 g $L^{-1}$), removed after a defined length of time, re-weighed and the water uptake calculated. Nasal inserts were prepared by lyophilising HPMC gel in 0.2 mL polythene microcentrifuge tubes.

*Dynamic adhesion test*

Lyophilised discs were prepared by freeze drying HPMC gel in blister-pack cavities using the cycle described above. Disc shapes were used for this test in order to exclude any potential variability due to the uneven 'torpedo' shape of the nasal insert, which may give a variable contact area that could affect adhesion measurements. It has previously been reported that inert substances may be used as a starting point for the investigation of mucoadhesion (Mortazavi & Smart 1995), and the use of agar as a test surface for bioadhesive materials has previously been described (Nakamura et al 1996, McInnes et al 2001, Bertram & Bodmeier 2006). Ultrapure agar at a concentration of 12 g $L^{-1}$ was prepared, cast into 25 cm × 25 cm agar plates, wrapped in cling film when cool, and allowed to set overnight. Lyophilised discs were placed near the top of the plate and held in contact with the surface of the agar with a force of 0.05 N for 60 s. The plate was then tilted to an 80° angle, allowing the hydrating discs to slide downwards, parallel to the surface. The distance travelled by a hydrating disc over time was measured.

Dynamic adhesion experiments were performed to assess the effect of the concentration of HPMC in the formulation, the molecular weight of the HPMC used, concentration of mannitol added to the formulation, and the addition of PVP in place of mannitol, with the aim of increasing the adhesive nature of the formulation.

*Texture analyser (TA) sliding adhesion*

A TA (XT2, Stable Micro Systems, Godalming, UK) was used in 'Measure force in tension' mode with a 5 kg load cell and a modified peel strength rig (Figure 1). Data gathered were analysed using the Texture Expert software (Stable Micro Systems, Godalming, UK). Lyophilised K4MP discs attached to glass sintered discs were prepared by filling HPMC gel into a mould containing the sinter. This produced a lyophilised substrate that could be permitted to hydrate and form an adhesive gel on the surface of the agar, while retaining a portion (the glass sinter) that would be rigid enough to enable the formulation to be pulled along the surface of the agar, allowing the TA probe to measure the force required to do this. HPMC gel was added to the device and allowed to penetrate into the pores of the sinter before lyophilisation, and enough gel was added to ensure that a unit was formed with lyophilised HPMC integrated with the glass sinter, with a portion protruding from the surface. The force required to slide hydrated HPMC discs parallel to the surface of the synthetic mucosal model (agar) was measured. This test was devised to overcome the problem that conventional TA methods tend to measure the force of adhesion by pulling the formulation perpendicular to the test surface, which is not representative of the forces acting on the formulation in the nasal cavity as the cilia slide the formulation along the mucosal surface.

*Statistical methods*

The data were analysed for statistical significance using one-way analysis of variance, using Minitab Release v14.1 (Minitab Inc., Coventry, UK). The effect of formulation type on the physicochemical properties derived from SEM, DVS, CSLM, dynamic adhesion test and TA sliding adhesion test were compared. Formulation performance at each particular time point was carried out for tests that included continual data recording. Following one-way analysis of variance, Tukey's test was performed to determine individual significance. A $P$ value below 0.05 was considered significant.

## Results and Discussion

### Scanning electron microscopy

The internal structure of the HPMC lyophilisate was sponge-like and highly porous, as shown in Figure 2. The honeycomb structure formed is most likely to be the result of the removal

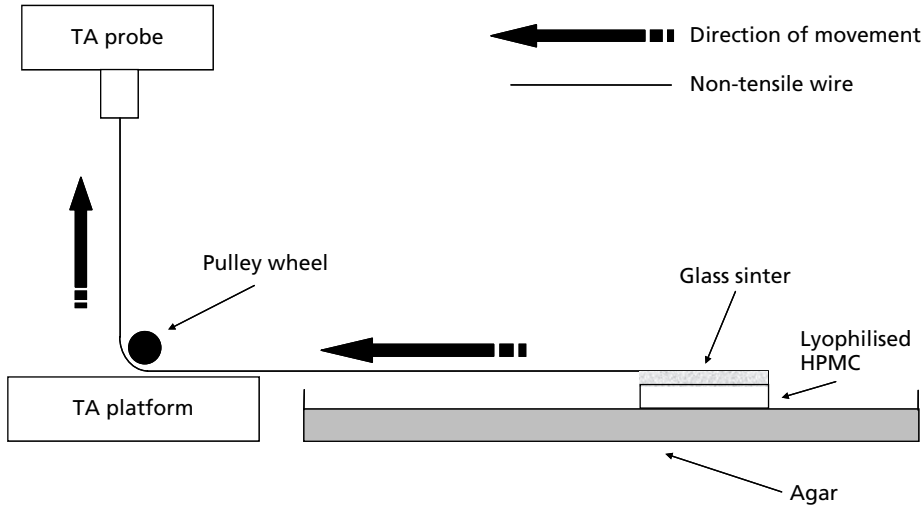


**Figure 1** Schematic of the texture analyser (TA) sliding adhesion test.

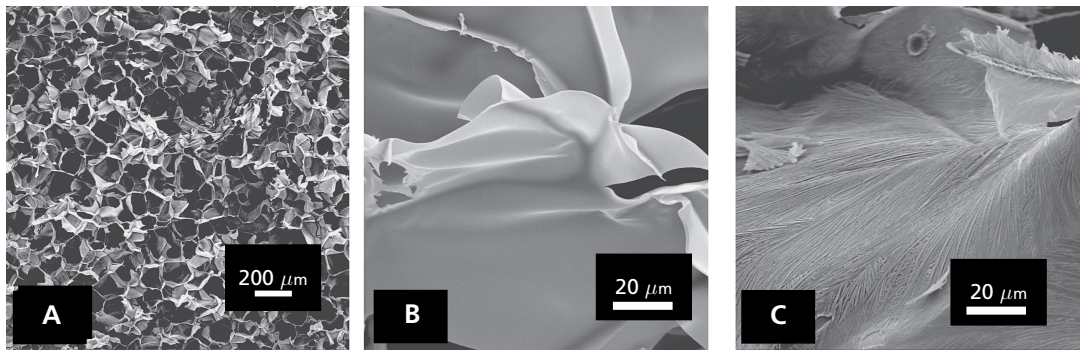


**Figure 2** Scanning electron microscope images showing the porous internal structure of a lyophilisate (A), the smooth 'leaf' surface of formulation D (B) and the presence of mannitol on the hydroxypropylmethylcellulose surface of formulation B (C).

of ice crystals during the sublimation step of lyophilisation, and would be expected to provide an ideal porous pathway for rehydration of the lyophilisate.

The mean pore size of the HPMC lyophilisates (formulations A–D) was determined by measuring a random sample of the pores (n = 10) on the SEM images (Table 2). Increasing HPMC concentration produced a decrease in the average internal pore size of the lyophilisate, suggesting that the higher HPMC concentration forces the porous network into a more compact form. This effect was not linear however, and showed signs of plateauing at a concentration of K4MP of 3%; reduction of available water space in the gels cannot be expected to reduce indefinitely. Lyophilisation of 2% K4MP with no mannitol resulted in a slight decrease in the pore size of the lyophilisate compared with that containing 1% mannitol, although this was not significant ($P > 0.05$).

Examination of the internal structure of the lyophilisates at higher magnification revealed that thin leaf-like structures surrounded the pores of the internal structure, which were completely smooth in the absence of mannitol (formulation D). Addition of mannitol (formulations A–C) resulted in a crystal needle structure imposed on the HPMC 'leaves' (Figure 2).

## Dynamic vapour sorption

The DVS analysis for formulations A–D demonstrated a substantial increase in weight as a result of sorption of water vapour, particularly in the phase of exposure to 95% RH (Table 2). Sorption capacity for formulations A–C ranged between 38% and 56% of the dry weight, lower than observed for formulation D, which did not contain mannitol. Previous DVS studies of mannitol powder demonstrated that mannitol alone has a very low sorption capacity compared with HPMC powder (data not shown). This may therefore be the factor that influenced the overall absorption capacity, particularly in the light of the SEM images which showed that the mannitol appears to remain on the surface of the HPMC in the lyophilisate. Formulation E, which contained a low-molecular-weight HPMC, absorbed a similar quantity of moisture as the higher-molecular-weight formulations, and formulation F, which contained PVP, had the highest moisture sorption capacity.

**Table 2** Effect of lyophilisate content on internal pore size, vapour sorption capacity, hydration parameters determined by confocal laser scanning microscopy and dynamic adhesion values (mean values ± s.d.)

| Formulation | Pore size ($\mu$m) (n = 10) | Vapour sorption capacity (% weight) (n = 1) | Initial hydration rate ($\mu$m s$^{-1}$) (n = 5) | Steady state hydration rate ($\mu$m s$^{-1}$) (n = 5) | Dynamic adhesion value (min cm$^{-1}$) (n = 5) | Area under curve (cm min) (n = 5) |
|---|---|---|---|---|---|---|
| A | 132.1 ± 31.4 | 37.83 | 52.25 ± 11.7 | 3.46 ± 0.53 | 32.65 ± 3.14 | 751 ± 170 |
| B | 91.14 ± 25.7 | 48.25 | 24.20 ± 3.70[a] | 0.98 ± 0.07[a] | 45.69 ± 1.45[a] | 1030 ± 246 |
| C | 75.00 ± 18.6[a] | 56.22 | 9.21 ± 5.40[a] | 0.41 ± 0.11[a,b] | 61.16 ± 2.97[a,b] | 1374 ± 396 |
| D | 84.78 ± 26.2 | 53.42 | 13.34 ± 3.2[a] | 0.70 ± 0.04[a] | 66.66 ± 3.23[a,b,c] | 1359 ± 356 |
| E | – | 52.52 | 55.13 ± 14.2[b,c,d] | – | – | – |
| F | – | 65.60 | – | – | 28.96 ± 2.37[b,c,d] | 801 ± 213 |

Data are mean ± s.d. [a]significantly different from A; [b]significantly different from B; [c]significantly different from C; [d]significantly different from D; all $P < 0.05$. For vapour sorption capacity, the coefficient of variation for all values was less than 7%.

## Confocal laser scanning microscopy

Before starting the studies, we demonstrated that the addition of fluoroscein did not alter the pore size of the lyophilised formulations, as determined by SEM, or affect dynamic adhesion values, and was therefore not expected to affect the rate of water ingress for CLSM studies.

A numerical value for the rate of water ingress into the lyophilisates was determined from individual images obtained using CLSM, by evaluating the distance moved by the water front over a defined period of time (n = 5). Water ingress at the edge of the lyophilisate was initially rapid but an apparent steady rate of water movement was reached after a few minutes. Therefore, we evaluated the initial rate of hydration over 10 s and the steady-state rate of hydration over 3 min (Table 2).

The initial rate of water ingress in the five formulations decreased in the order E > A > B > D > C. The order was the same for the steady-state rate of water ingress, with the exception of formulation E, which had completely hydrated by this time and therefore could not be assessed. The steady-state hydration rate was calculated to be 1–7% of the initial instantaneous hydration rate. Representative CLSM images demonstrating the differences observed in hydration rate are shown in Figure 3.

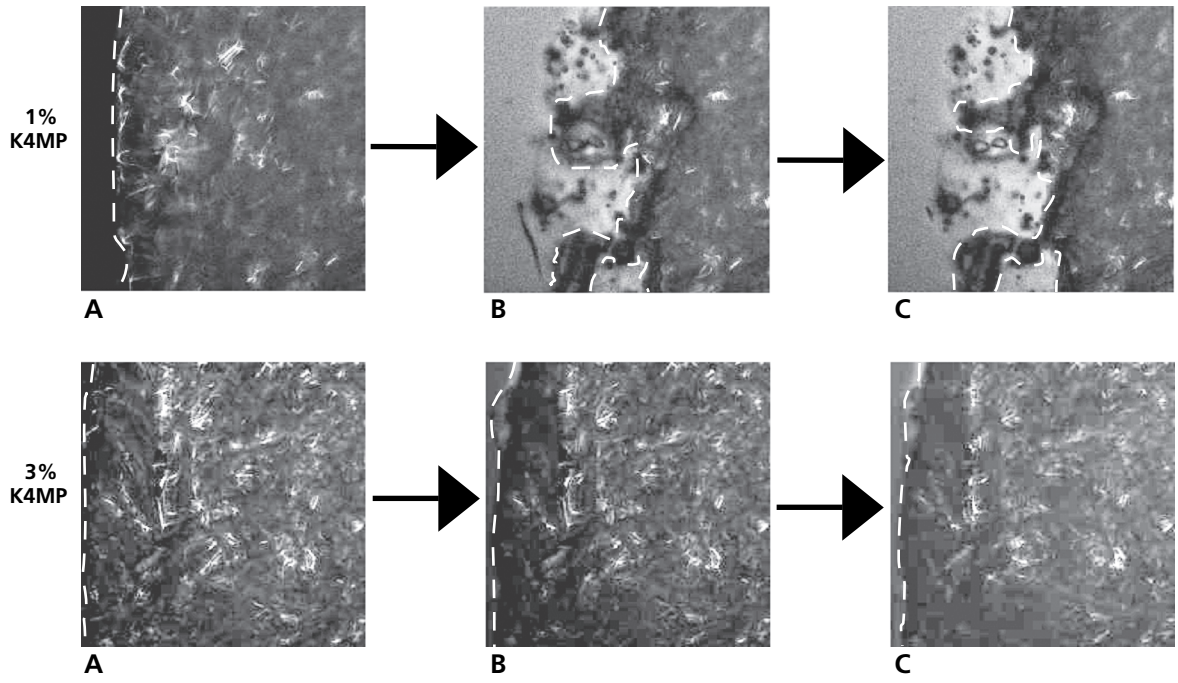


**Figure 3** Confocal laser scanning microscopy images of 1% and 3% K4MP hydroxypropylmethylcellulose lyophilisates at 0 s (A) 4 s (B) and 8 s (C) after addition of water. The broken lines mark the progressing water front moving from left to right across the image and the hydrating lyophilisate.

*Simple hydration test*

All formulations absorbed the equivalent of more than 10 times their weight in water during the course of the study period (Figure 4). Initial hydration (15 min) was rapid, and mean increase in weight due to water intake was 2–5 times that of the dry weight. The rate of hydration in the first 15 min decreased in the order D > A > E > F > B > C, showing that the rate of hydration was decreased by HPMC concentration, HPMC molecular weight, addition of mannitol and addition of PVP (in place of mannitol). Addition of 1% PVP to HPMC resulted in a less pronounced decrease in hydration rate than the addition of 1% mannitol. Following 1 h of hydration, the order of rate of hydration was more or less the same as that for the initial rate. This is the time period considered to be crucial in an in-vivo context, as initial hydration must be rapid in order for the formulation to gel and adhere to the nasal mucosa. At the end of the 5 h study period, the total water absorbed by each formulation decreased in the order D > B > C > A > F > E, and total water uptake for formulations A–D appeared to be approaching a plateau. This may mean that these formulations rapidly approach a state of maximum hydration, which may be of importance for a formulation that is designed to reside in the nasal cavity for an extended period of time. At the 5 h time point, formulation A had absorbed significantly more water than formulations B and D ($P < 0.05$); formulation B had absorbed more than formulations C, D, E and F ($P < 0.05$); formulation C had absorbed more than formulations D and E ($P < 0.05$); formulation D had absorbed more water than formulations E and F ($P < 0.05$).

## Dynamic adhesion test

The distance travelled by a hydrating lyophilisate over a known time period was measured, resulting in a reproducible dynamic adhesion profile (Figure 5). During the test, initial sliding of the K4MP formulations was slow, and the rate of movement increased as the formulations hydrated. The square root of distance plotted against time produced a straight line from which a dynamic adhesion value (taken as the inverse of the slope) could be obtained as a quantitative measure of adhesion. The area under the curve (AUC) was also calculated to quantify the total work of adhesion. The effect of varying the composition of the lyophilisates is shown in Table 2. Increasing HPMC concentration resulted in an increase in the dynamic adhesion value and AUC. The low-molecular-weight HPMC in formulation E produced a lyophilisate with such low adhesion that no data could be collected as it had hydrated and travelled to the bottom of the test surface before the first measurement. The HPMC formulation without mannitol (D) had a higher adhesion value than the equivalent formulation containing mannitol (B; $P < 0.05$), suggesting that the rapid hydration of this formulation observed in the simple hydration test may contribute to the greater adhesion observed in this test.

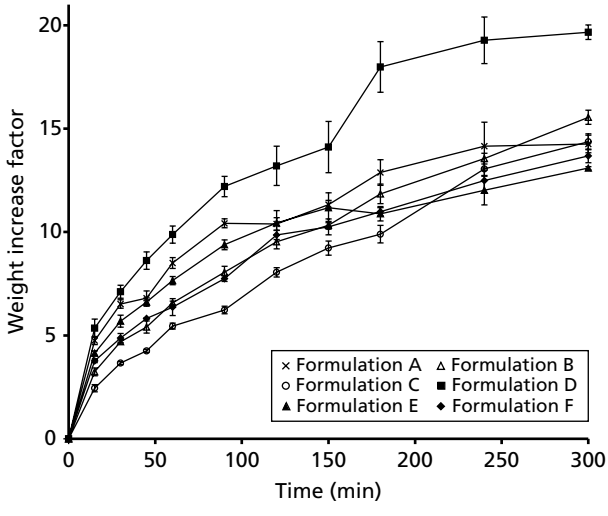


**Figure 4** Simple hydration profiles of lyophilised formulations; data are mean ± s.d.; n = 5.

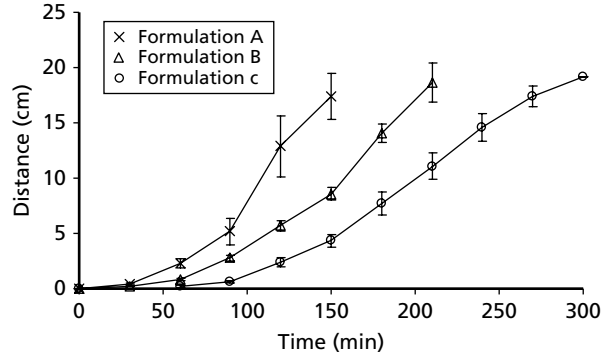


**Figure 5** Representative dynamic adhesion profiles of formulations A, B and C; data are mean ± s.d.; n = 5.

Addition of PVP polymer to the HPMC formulation would generally be expected to increase adhesion (Jones et al 2002). However, the dynamic adhesion value for formulation F was lower than that for the equivalent mannitol-containing lyophilisate (B; $P < 0.05$). These findings are similar to those of Tobyn et al 1996, who found that inclusion of PVP in mucoadhesive tablets significantly reduced adhesion, and Chan et al 2003, who reported an anti-tack action of PVP on HPMC solutions. The authors suggested that as PVP polymers are hydrogen-bond acceptors, they preferentially bond with hydrated polymers, reducing the degree and strength of interaction between the mucoadhesive and the mucus in gastric tissue, and the tack of HPMC. In our study we observed that the formulation containing PVP remained only partially hydrated at the area of contact with the agar for an extended period of time, further suggesting that this formulation was unable to hydrate efficiently. This suggestion is supported by the low ranking of this formulation (F) in the simple hydration test.

*Texture analyser sliding adhesion test*

Formulations A–C were studied using the TA. The peak force required to overcome adhesion was recorded as the peak initial force required to slide the formulation, and the AUC was calculated as a measure of the work done to overcome adhesion (Table 3). Initial adhesion of the formulations was high (Figure 6), although adhesion of the 3% K4MP formulation

**Table 3** Effect of lyophilisate formulation on texture analyser sliding adhesion

| Formulation | Initial | | 60 min | | 240 min | |
|---|---|---|---|---|---|---|
| | Peak force (N) | AUC (N s) | Peak force (N) | AUC (N s) | Peak force (N) | AUC (N s) |
| A | 0.49 ± 0.04 | 2.10 ± 0.27 | 0.14 ± 0.02 | 0.86 ± 0.17 | 0.05 ± 0.00 | 0.26 ± 0.04 |
| B | 0.54 ± 0.05 | 1.83 ± 0.19 | 0.30 ± 0.07[a] | 1.79 ± 0.42[a] | 0.11 ± 0.01[a] | 1.08 ± 0.05[a] |
| C | 0.20 ± 0.02[a,b] | 1.01 ± 0.29[a,b] | 0.29 ± 0.03[a,b] | 0.99 ± 0.34[b] | 0.14 ± 0.01[a,b] | 0.99 ± 0.06[a] |

Values are mean ± s.d.; n = 6. [a]significantly different from A; [b]significantly different from B ($P < 0.05$).

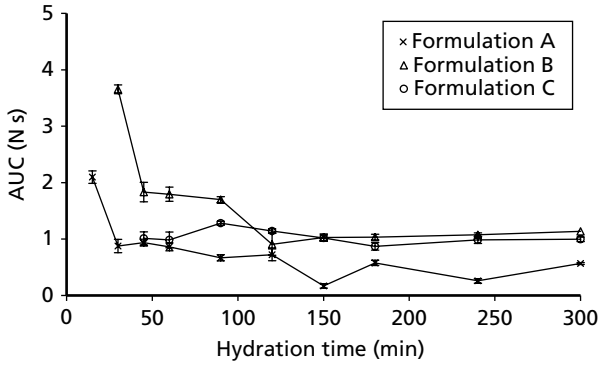


**Figure 6** Effect of extent of hydration on area under the curve (AUC) of K4MP hydroxypropylmethylcellulose formulations in the texture analyser sliding adhesion test. Data are mean ± s.d.; n = 6.

could not be recorded before 45 min, as it had not hydrated sufficiently to adhere to the agar synthetic mucosal surface. Adhesion was reduced as the formulations became increasingly hydrated, and tended to plateau after approximately 2 h.

Overall, the 2% K4MP formulation displayed the optimal combination of adhesion in the TA sliding adhesion test: it had high initial adhesion, and adhesion at 60 min was still significantly higher than that of formulations A and C ($P < 0.05$). Although in the latter stages, the 3% K4MP lyophilisate displayed similar adhesion to formulation B, lack of initial adhesive effect would be a drawback in-vivo, where only a short period of time would be available for a lyophilisate to establish initial adhesion.

Formulation parameters that increase water ingress into the lyophilisates are likely to result in a more rapid adhesive effect through formation of the polymer gel. In particular, initial water ingress is important (Hedenus et al 2000), as it is this surface that provides an interface with the mucosal surface and is involved in forming initial adhesive bonds. Conversely, if the initial rate of hydration is too high, over-hydration may occur, resulting in a lack of useful adhesive properties (Mortazavi 1995). The importance of the initial rate of hydration is clear from the results of the TA sliding adhesion test, where the 3% K4MP formulation displayed a lack of initial adhesion because of lack of hydration.

The apparent general decrease in adhesion on the TA sliding adhesion test after 90 min further demonstrates the effect of over-hydration, as does the gradual decrease in adhesion after approximately 90 min during the dynamic adhesion test. The lack of adhesion demonstrated by the rapidly hydrated K100LV formulations in the dynamic adhesion test also demonstrates the effect of over-hydration of the formulations on adhesive capabilities. This effect is similar to that described in studies which found that increasing water content of Carbopol 934P gels decreased adhesiveness and cohesion (Henriksen et al 1996; Mortazavi & Smart 1993), where it was concluded that water uptake might be of more relevance to adhesion than surface molecular interactions. A further study concluded that an increased rate of swelling resulted in a decreased duration of adhesion (Mortazavi & Smart 1994).

## Discussion

Each of the methods reported provided useful information on the hydration and adhesion properties of the lyophilisates, and allowed comparison between different formulations. However, as many of the properties reported here were expected to display some interdependent behaviour, the data gathered were then considered as a whole, in order to discern any relationships between the various parameters. A correlation was observed between internal lyophilisate pore size (SEM) and initial/steady-state hydration rate (CLSM) ($R^2 = 0.984/0.989$), which may be in agreement with the findings of Hedenus et al (2000), who reported that cellulose powder porosity and instantaneous absorption capacity correlate in a manner consistent with the idea that the absorption of water is via a capillary process. In the current study it was also noted that for K4MP formulations (A–C), the extent of hydration at 15 min of simple hydration correlated with the internal pore size and initial hydration rate measured by SEM ($R^2 = 0.995$) and CLSM ($R^2 = 1$), respectively, further suggesting a link between pore size and hydration rate of the formulations. However, this correlation did not extend to the other formulations in this study, suggesting that the hydration of the lyophilisates may not depend solely on these parameters. Likewise, it was not possible to determine any correlation between pore size and dynamic adhesion values, between rate of hydration determined by CLSM and dynamic adhesion values, or between simple hydration profiles and dynamic adhesion. This suggests a complex interplay between different factors that determine adhesion, although in the case of CLSM correlation, this may have been made difficult because of the rapid initial hydration rates (in the order of seconds);

such rapid and detailed measurement was not possible in the 'manual' dynamic adhesion test.

In the dynamic adhesion test, adhesion of the lyophilisates increased with HPMC polymer concentration ($P < 0.05$). However, the TA sliding adhesion test suggests that too high a polymer concentration may be detrimental to the initial adhesive effect, most likely because of the resulting impedance of the compact polymer matrix on water ingress and hydration, as observed in the CLSM and simple hydration tests. The lack of expression of this effect in the dynamic adhesion test demonstrates that using only one type of adhesion test can produce potentially misleading results.

During the course of the CLSM study, an instantaneous 'flash' effect was occasionally observed on addition of water. We hypothesise that this results from the mannitol in the formulation being rapidly dissolved and providing an osmotic pathway to conduct a very small amount of water through the polymer matrix, while not being of sufficient quantity to result in gel formation. In the case of formulations A–C, the pore size of the lyophilisate correlated with the initial rate of hydration ($R^2 = 0.9969$), suggesting that the pore size may have some influence on the rate of water ingress into the formulation.

Hydration kinetics of lyophilised formulations have a direct influence on adhesivity, and are therefore of paramount importance when designing bioadhesive formulations. Ideally, a combination of hydration tests – as described in this report – would be used to assess the properties of prospective formulations to obtain an overall picture of the hydration/adhesion process, removing individual limitations of a single method. For example, the main drawback of the TA sliding adhesion test is that while it can quantify forces of adhesion after a specific period of hydration, it does not account for the spreading and movement of the gel in the nasal cavity by the cilia, an effect better represented in the dynamic adhesion test. Other researchers have also presented a collection of different techniques to create an overall picture of the processes taking place (Mortazavi & Smart 1994; Jones et al 1997; Eouani et al 2001), and others have reported the limitations of using only one technique to study mucoadhesion (Hägerström & Edsman 2003).

In this study, agar was used as a synthetic mucosal surface, designed to provide a reproducible uniform surface with a defined amount of water available for hydration. This avoided any variability that may be encountered when using excised mucosal tissue, for example in the thickness, springiness or mucus composition and quantity. However, in future it may be of value to add homogenised mucus to the agar test surface, to better represent the situation in-vivo.

An ideal bioadhesive formulation is most likely to be one that displays an optimal combination of properties in the above tests, rapid initial hydration and therefore adhesion, without becoming over-hydrated, which would result in loss of adhesion.

## Conclusion

A range of techniques were developed to assess the in-vivo hydration and adhesion properties of lyophilised nasal formulations, and was intended to represent the dynamic processes occurring in the nasal cavity. The limitations of individual in-vitro adhesion tests in quantifying all aspects of in-vivo bioadhesion must be considered when evaluating lyophilised formulations, and the current studies demonstrated the possibility of obtaining misleading results if only one type of test is used. It is therefore advisable to use a range of techniques to evaluate different aspects of the hydration/adhesion process in order to fully evaluate a nasal bioadhesive formulation.

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