ABSTRACT

Objective: An aqueous-organic precipitation technique was developed for preparing bioadhesive hydroxypropyl methylcellulose (HPMC) microparticles for nasal delivery using a low viscosity grade of HPMC K100LV.

Methods: The method involved the preparation of HPMC microparticles by precipitating the gelled HPMC into acetone. The effect of different processing variables of precipitation technique on in-vitro bioadhesive performance of HPMC microparticles was studied.

Results: In-vitro assessment of adhesion properties of the microparticles showed that the process of co-precipitation greatly increased the adhesiveness in comparison with unprocessed HPMC powder. Bioadhesive performance was found to be dependent on the dehydrated state of the polymer following precipitation, with the most dehydrated formulations using the slowest (dropwise) rate of addition of the HPMC gel to acetone, showing lower water uptake and greater adhesion performance. It would be expected therefore, that the interaction of acetone and water would be instantaneous and complete, resulting in an extremely efficient dehydration process and a collapse of HPMC matrix structure, forming a physical barrier to subsequent rehydration.

Conclusion: This formulation afforded the highest bioadhesion score in the test employed, which was designed to represent the dynamic interplay of hydration, entanglement of polymer and mucin, and rheological flow which a formulation would experience on the nasal mucosal surface.

Keywords: Bioadhesive microparticles, Water sorption, Nasal drug delivery, Dynamic adhesion, Co-precipitation technique, HPMC polymer, Differential scanning calorimetry.

INTRODUCTION

The nasal route is considered an attractive alternative to parenteral administration, for reasons which include direct entry to the systemic circulation for nasally administered drugs, avoidance of first-pass metabolism, the high total blood flow and a large mucosal surface area providing ideal conditions for absorption[11].

Mucociliary clearance is the main drawback of this route, clearing formulations from the nasal cavity in approximately 21 minutes [2,3]. The use of bioadhesive delivery systems promotes adhesion of the formulation to the nasal mucosa, allowing an extended period of contact for drug absorption to occur. Bioadhesive polymers such as cellulose derivatives have the ability to absorb water and swell as a result of their hydrophilic nature, and the swollen polymer chains can then interact and tangle with the glycoproteins of mucin by hydrogen bonding, altering the properties of the mucous layer and reducing the rate of mucociliary clearance [4,5]. Excessive hydration can be detrimental[6-8], and lead to decreased mucin adhesion and/or retention due to the formation of smooth mucilage [9]. A wide range of literatures reported the preparation of bioadhesive microparticles for nasal delivery using either spray drying[23,24], solvent evaporation[25-27] or coacervation /phase separation[28-30]. However, co-precipitation techniques have not been widely reported and the basis of this work was to evaluate a precipitation technique to produce bioadhesive polymer microparticles as the basis for future development as co-precipitated drug-polymer formulations. The choice of the solvents used for HPMC precipitation and their ratio with water was taken into account since this polymer has certain solubility in aqueous-organic solutions[31,32]. Previous literature reports have suggested that the low viscosity grade of HPMC, K100LV showed poor bioadhesive performance, and it was hypothesized that this performance could be improved by utilization of appropriate processing methods. Different analytical methods were used to identify the physicochemical changes of HPMC in processed microparticles in comparison to unprocessed polymer, and explain their resultant performance as a bioadhesive formulation.

MATERIALS AND METHODS

Materials

HPMC powder (K100LV) was a gift from Dow Chemicals Ltd, Michigan, USA. Acetone and porcine stomach mucin type II was purchased from Sigma-Aldrich, Dorset, UK. Ultra-pure agar and Parafil® were purchased from VWR, Poole, UK. Distilled water was produced in house.
Methods

Preparation of HPMC bioadhesive microparticles

Initially, an aqueous gel of HPMC was prepared in a concentration of 4% w/w. The appropriate weight of HPMC was added to approximately one third of the final amount of distilled water (DW) required, maintained at a temperature of 80-90°C. The mixture was stirred until a uniform solution was obtained, following which the remaining water was added. The solution was again stirred until a uniform gel was obtained. The resultant gel was stored at 4°C overnight before use to allow air bubbles to dissipate.

A 10 ml volume of HPMC gel was dropped into 200 ml of the precipitating agent, acetone in a ratio of 1:20, at a rate of 10 ml/hr using a syringe pump (SP, Cole-Parmer, London, UK) with a 23 gauge needle. During addition of the HPMC gel, the precipitating agent was agitated by a high shear mixer Silverson SL 2T (Silverson, Waterside, UK) using an 8000 rpm stirring speed for one hour to obtain microparticles (formulation B, Table 1). The beaker used for preparation was covered with Parafilm during dropping, in order to prevent evaporation of the precipitating agent. Microparticles were collected by filtration using a Buchner funnel and water vacuum. Following filtration, the sample was left in a fume hood until dry before being placed in a vacuum oven for 6 hours to evaporate any residual solvent entrapped within the microparticles. The dehydrating effect of the precipitating agent (acetone) on the aqueous HPMC gel was assessed using different volumes of acetone as shown in Table 1, to study the effect on the physical properties of the resultant HPMC microparticles.

In-vitro dynamic adhesion

In order to examine the mucoadhesive potential of the HPMC microparticles, an artificial nasal mucosa was prepared using a concentration of 1% agar and 2% mucin w/v according to methods described elsewhere [33,34]. Separate agar and mucin solutions were prepared, following which the agar solution was left to cool down to 50°C before adding the mucin solution with stirring. The mixture was then rapidly poured into plates of 25×25 cm and left for one hour to set. Following this, the plates were wrapped with cling film to prevent any change in the plates were re-wrapped with cling film to prevent any change in the temperature overnight to standardize the media to be used for dynamic adhesion measurements the following day. 30 g of HPMC powder or microparticles were placed at the top of the plates. Then, a force of 0.05 N was applied to each sample for 60 seconds. All plates were re-wrapped with cling film to prevent any change in the humidity or drying out of the adhesion medium environment for the duration of the experiment. The plates were tilted to an angle of 80° and the samples allowed sliding down the plate, representing the direction of movement within the nasal cavity [33,34]. The distance travelled by the samples with time was measured and used as an indication of extent of bioadhesion. The inverse of the slope of the resultant distance versus time curve was used as an indicative value of the extent of bioadhesion of all formulations in Table 1 [16].

Table 1: Processing technique variables for preparation of HPMC microparticles

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Concentration of HPMC (%w/w)</th>
<th>Volume of acetone (ml)</th>
<th>Technique used</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HPMC</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>200</td>
<td>GAH</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>100</td>
<td>GAH</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>50</td>
<td>GAH</td>
</tr>
<tr>
<td>E</td>
<td>4</td>
<td>200</td>
<td>DAM</td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>100</td>
<td>DAM</td>
</tr>
<tr>
<td>G</td>
<td>4</td>
<td>50</td>
<td>DAM</td>
</tr>
<tr>
<td>H</td>
<td>4</td>
<td>200</td>
<td>DAH</td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td>100</td>
<td>DAH</td>
</tr>
<tr>
<td>J</td>
<td>4</td>
<td>50</td>
<td>DAH</td>
</tr>
<tr>
<td>K</td>
<td>4</td>
<td>200</td>
<td>NG</td>
</tr>
<tr>
<td>L</td>
<td>400 mg</td>
<td>200</td>
<td>DAM</td>
</tr>
</tbody>
</table>

GA = Gradual addition, DA = Direct addition, NG = Non gelled, H = High shear mixing, M = Low shear mixing.

Acetone volume: The dehydrating effect of the precipitating agent (acetone) on the aqueous HPMC gel was assessed using different volumes of acetone as shown in Table 1, to study the effect on the physical properties of the resultant HPMC microparticles.

Effect of preparation technique

Table 1 summarizes the formulation and processing variables studied. The effect of agitation force in the precipitation step on the performance of the resultant microparticles was examined by replacing the high shear method (H) described in 2.2.1 with a low shear magnetic stirrer (M) (BibbySterilin Ltd, Staffordshire, UK).

The effect of direct addition (DA) of the entire HPMC gel to the precipitating agent rather than gradual addition using a syringe pump (GA, preparation of HPMC microparticle section), using either high or low shear mixing was studied.

A non-gelled (NG) formulation was prepared by immediate addition of acetone to HPMC powder sprinkled on water, to study the role of the hydrated gel in the properties of the resultant microparticles. HPMC powder was also directly added to acetone in the absence of water, using a low shear magnetic stirrer, to examine further the role of water in the formulations.

Formulations. All formulations were stored in an airtight sealed container until further analysis. The percent yield of all formulations was calculated using Equation 1.

\[
\% \text{ Yield} = \frac{\text{Actual dried weight of microparticles}}{\text{Theoretical weight}} \times 100
\]

Scanning electron microscopy (SEM)

SEM images were obtained for HPMC powder (A) and 4% HPMC microparticle formulations (B, E, H and K, Table 1) to study the effect of different preparation variables and water addition on the morphology of HPMC in the microparticles. Samples were prepared by sprinkling on 10 mm aluminum stubs mounted with double sided copper tape. Samples were coated with gold using a sputter coating system (Polaron SC 515, Ashford, UK). Observations were made with a JEOL 6400 SEM operating at 6KV using the secondary electron mode.

Differential scanning calorimetry (DSC)

DSC (DSC822e Model; TA controller TC15, Mettler-Toledo Ltd, Leicester, UK) was used to study the effect of process and formulation variables described in Table 1 on the thermal behavior of the resultant HPMC microparticles in comparison to HPMC powder. A method described by Okhamafe and York (1985), was used to obtain an accurate measurement of the glass transition temperature (Tg) of HPMC.

Samples were exposed to a heating rate of 15-125°C at 10°C/min and then held for 10 minutes at 125°C to remove any moisture from the sample that may obscure the Tg. Afterwards, the sample was quenched cooled to -40°C and held at this temperature for 5 minutes before heading to 250°C at a rate of 20°C / min [35]. The software (Star system, Mettler-Toledo Ltd, Leicester, UK) was used to calculate the Tg and integrate the endothermic peak between 20-120°C.
Dynamic vapor sorption (DVS)

Dynamic vapor sorption (Surface Measurement System, SMS Ltd., Alperton, UK) was used to study the effect of the processing techniques described in Table 1 on the ability of HPMC microparticle formulations to absorb water, and important property in situ in order to rapidly form a gel which can overcome mucociliary clearance.

Samples weighing 8-10 mg were loaded on to the pan and the program was set to control the humidity at 0% RH, followed by increasing RH in 10% increments to 95%. The RH was then decreased through the same steps, and the temperature maintained at 25 ±0.5°C throughout the cycle. A sorption/desorption profile was obtained from the DVS software (Surface Measurement System, SMS Ltd., Alperton, UK), and the overall mass increase of the sample (in relation to the dry weight) at 95% RH was calculated to assess the extent of water vapor sorption.

Nuclear magnetic resonance spectroscopy (NMR)

$^1$H-NMR (Delta V 4.3,3,BCX-400MHz, Jeol, Tokyo, Japan) was used to elucidate any chemical shift, solvent peak (acetone) and number of protons or hydrogens (H-bonding groups) of HPMC formulations in Table 1 in comparison to unprocessed HPMC powder as a result of aqueous-acetone co-precipitation techniques. 20 mg of solid samples were weighed and left sealed overnight to complete their dissolution. The solutions were transferred into NMR tubes to be analyzed using the NMR apparatus. A pre-saturation method of single pulse was used for the analysis.

Statistical analysis

A T-test was used for two samples of equal variances, to analyze the significant differences among formulations by changing one factor. This test was used for all analytical studies in this work. The differences were considered significant when the probability was $P \leq 0.05$.

Results and discussion

Physical properties of microparticles

The percent yield of formulations prepared by precipitating 10 mls of HPMC solution precipitated in the highest volume of acetone, 200 ml, was 80-90%. When lower volumes of acetone, 100 and 50 ml were used the yield decreased to 60-70%. This suggests that a larger proportion of the HPMC gel remained soluble in the aqueous-organic mixtures in solvent ratios less than 1:20.

The physical appearance of the precipitated microparticles was visually different depending on the processing method used. The HPMC in aqueous-acetone medium produced discrete microparticles, whereas the GAH method resulted in hard microparticles that aggregated to form macroparticles. The DAM method produced film like structures of aggregated macroparticles. The DAH technique produced material with characteristics of both of hard microparticles that aggregated to form macroparticles and film like structures of aggregated macroparticles. For formulation K where HPMC only had transient contact with water prior to addition of acetone (NG), a mixture of fine particles and sheet like structures were produced.

In-vitro dynamic adhesion

The effect of preparation method (GAH, DAM or DAH) on the dynamic adhesion of the processed polymer is shown in Fig. 1. The inverse slope of the linear part of adhesion curves in Fig. 1 was used for quantification of the bioadhesive potential.

The dynamic adhesion of processed HPMC microparticles was higher than for HPMC powder. This suggests that the process of dropping or mixing HPMC aqueous gel directly into acetone and subsequent drying produced this increase in the bioadhesive effect. However, particle size and morphology may also play a role, since the above studies compare discrete particles of HPMC powder, with aggregated materials produced on processing. The dropping technique (GAH) applied in formulation B resulted in higher in-vitro bioadhesion in comparison to the other two methods of precipitation DAM (formulation E), and DAH (formulation H).

The dynamic adhesion profiles of HPMC microparticle formulations E, F and G (DAM, using different volumes of acetone), and compared with HPMC powder are illustrated in Fig. 2.

As the volume of precipitating agent increased, the dynamic adhesion increased significantly. This suggests that the hydrophilic nature of acetone caused rapid dehydration of the HPMC aqueous gel according to its volume [36].

Fig. 3 summarizes the effect of the different processing variables on the dynamic adhesion of all HPMC formulations, as the adhesion increases the 1/slope value increases.

To examine the effect of water and hydration of HPMC prior to addition to acetone, HPMC powder was treated with acetone in the absence of water, and also in a process allowing partial hydration by sprinkling HPMC onto water followed by rapid quenching with acetone as described in the effect of preparation technique section. The dynamic adhesion data for these variables are shown in Table 2.

Treating HPMC powder with acetone afforded an uncertain enhancement in adhesion properties, while transient contact with water in formulation K produced a significant increase over HPMC powder, although not of the magnitude of material from the precipitation process using dropping technique (GAH, formulation B). It is hypothesized that the presence of water initially with HPMC, before addition to acetone was important to modify the bioadhesion.
HPMC molecules have the ability to absorb water due to the OH groups in its structure. With the completely gelled HPMC, there is a complete network of water surrounding the polymer. In such network, a water molecule may concurrently form hydrogen bonds with two or more polar groups on the polymer surface, in that way becoming highly immobilized [37].

It was proposed that precipitation with acetone caused complete and rapid dehydration of this system, effectively immobilizing the expanded steric structure of the HPMC, leaving hydrogen bond forming moieties exposed for subsequent interaction with water and/or mucin.

**Scanning electron microscopy**

Representative SEM images of HPMC powder and HPMC microparticles precipitated by acetone using different processing variables are shown in Fig. 4. The HPMC microparticles were morphologically dissimilar, depending on the method of preparation employed.

**Table 2: In-vitro dynamic adhesion values HPMC microparticles, n=6**

<table>
<thead>
<tr>
<th>Formulations</th>
<th>HPMC forms</th>
<th>Precipitation method, 200ml acetone</th>
<th>1/Slope (min/cm)</th>
<th>Regression (r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Powder alone</td>
<td>DAM</td>
<td>4.60 ±0.06</td>
<td>0.9401</td>
</tr>
<tr>
<td>K</td>
<td>Powder sprinkled on water</td>
<td>NG</td>
<td>9.35 ±1.3*</td>
<td>0.9862</td>
</tr>
<tr>
<td>B</td>
<td>Aqueous gel</td>
<td>GAH</td>
<td>15.85 ±4.1*</td>
<td>0.9912</td>
</tr>
</tbody>
</table>

* P value ≤ 0.05
Different matrices were formed according to the precipitation technique used. Dropping of HPMC aqueous gel into acetone using H mixer (GAH method) formed a continuous structure as shown for formulation B. A similar structure was observed for formulation H, although slightly smoother in appearance, when HPMC aqueous gel was added to acetone using the same mixer (non-dropping, DAH method).

While mixing of HPMC aqueous gel with acetone (non-dropping) using M stirrer (DAM method), produced a smooth sheet like structure as shown for formulation E. HPMC microparticles (formulation K) which involved the addition of acetone directly to HPMC sprinkled on water (NG method) using M stirrer produced a little difference in places by maintaining a more open polymer structure as unprocessed HPMC powder.

The smallest quantity of water was detected in microparticles formed by the GAH method, where it was noted that as the volume of acetone used increased, the efficiency of water removal also increased. A similar trend for acetone volume was observed for both the DAM and DAH methods, although neither was as efficient as the GAH method. Direct addition of HPMC powder to acetone, produced no change in water content compared with untreated HPMC powder, while the technique employing transient exposure to water followed by acetone, revealed a modest decrease in water content when compared to HPMC alone. Formulations B, C and D prepared by GAH method using 200 ml, 100 ml and 50 ml acetone respectively, produced integrated peaks of 169.88 mJ, 230 mJ and 279 mJ and Tg values of 187.7 °C, 182.66 °C and 179 °C respectively. This is may be a result of the dehydration effect of acetone causing a decrease in the water content and increasing the Tg of the HPMC matrix [40].

Water uptake measurement using DVS

Moisture uptake was studied in order to further understand the bioadhesion behavior of processed microparticle formulations shows the correlation between acetone volume, processing method and resultant maximum vapor sorption at 95% RH using DVS assessment.

It was observed that the sample containing the least water following processing (formulation B), demonstrated the lowest capacity for water uptake during the study. This result was unexpected as it would be supposed that the more dehydrated formulation would be more susceptible to water uptake[5,41].

Equally important are the data points for 200 ml acetone of formulations B (GAH method), E (DAM method) and H (DAH method), where the effect of processing on vapor sorption capability of the particles are striking, and the previous "water history" of HPMC appears to dictate the capability to uptake moisture. For example, direct addition of acetone to HPMC powder (formulation L, DAM method) reduces its vapor sorption potential in comparison to unprocessed HPMC powder.

If HPMC is fully hydrated as a gel before forming microparticles, then the vapor sorption capability is further reduced, and is dependent on the efficiency of the dehydration process with acetone. Mixing and method of addition of components therefore has a pronounced effect. However, this finding was not the same as other reports, in which the fully hydrated HPMC as a gel before dehydration using lyophilization displayed either more increment in mass [42] or absorbed the same water vapor (over 50%) of its dry weight when compared to HPMC powder [43]. Therefore the current finding requires further study using NMR which will be performed in the following section [44].

Differential scanning calorimetry

HPMC has a broad endothermic peak between 20-110 °C, and the evaporation of water can be identified by DSC between 80-125°C. Therefore any increase in the depth of the endothermic peak at 20-125 °C can be helpful in determining the level of hydration or dehydration of the processed polymer. This method was therefore used to quantify the degree of dehydration caused by acetone[38]. Also, the glass transition temperature (Tg) of the polymer can be used for the same purpose given that the presence of entrapped water molecules (bounded by H-bonding) acts as a plasticizer that decreases Tg of polymer[39]. These parameters were measured to evaluate the effect of acetone on the hydration state of the processed polymer. Table 3 summarizes the data obtained from the integrated peak between 20-125°C and Tg determination.

Table 3: DSC results of HPMC powder and microparticle formulations

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Precipitation method</th>
<th>DSC Integrated peak 20-125°C (mJ)</th>
<th>Glass transition temperature (Tg °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMC (A)</td>
<td>-</td>
<td>697.81</td>
<td>168.5</td>
</tr>
<tr>
<td>B (gelled HPMC, 200ml acetone)</td>
<td>GAH</td>
<td>169.88</td>
<td>187.7</td>
</tr>
<tr>
<td>C (gelled HPMC, 100ml acetone)</td>
<td>GAH</td>
<td>230</td>
<td>182.6</td>
</tr>
<tr>
<td>D (gelled HPMC, 50ml acetone)</td>
<td>GAH</td>
<td>279</td>
<td>179</td>
</tr>
<tr>
<td>E (gelled HPMC, 200ml acetone)</td>
<td>DAM</td>
<td>300.81</td>
<td>179</td>
</tr>
<tr>
<td>F (gelled HPMC, 100ml acetone)</td>
<td>DAM</td>
<td>344.64</td>
<td>176</td>
</tr>
<tr>
<td>G (gelled HPMC, 50ml acetone)</td>
<td>DAM</td>
<td>350.12</td>
<td>172</td>
</tr>
<tr>
<td>H (gelled HPMC, 200ml acetone)</td>
<td>DAH</td>
<td>233.53</td>
<td>185.1</td>
</tr>
<tr>
<td>I (gelled HPMC, 100ml acetone)</td>
<td>DAH</td>
<td>233.76</td>
<td>182.4</td>
</tr>
<tr>
<td>J (gelled HPMC, 50ml acetone)</td>
<td>DAH</td>
<td>305.66</td>
<td>181</td>
</tr>
<tr>
<td>K (HPMC powder+10mldW+200mlacetone)</td>
<td>NG</td>
<td>496.66</td>
<td>176.7</td>
</tr>
<tr>
<td>L (HPMC powder+200 ml acetone)</td>
<td>DAM</td>
<td>692.01</td>
<td>168.6</td>
</tr>
</tbody>
</table>

The smallest quantity of water was detected in microparticles formed by the GAH method, where it was noted that as the volume of acetone used increased, the efficiency of water removal also increased. A similar trend for acetone volume was observed for both the DAM and DAH methods, although neither was as efficient as the GAH method. Direct addition of HPMC powder to acetone, produced no change in water content compared with untreated HPMC powder, while the technique employing transient exposure to water followed by acetone, revealed a modest decrease in water content when compared to HPMC alone. Formulations B, C and D prepared by GAH method using 200 ml, 100 ml and 50 ml acetone respectively, produced integrated peaks of 169.88 mJ, 230 mJ and 279 mJ, and Tg values of 187.7 °C, 182.66 °C and 179 °C respectively. This is may be a result of the dehydration effect of acetone causing a decrease in the water content and increasing the Tg of the HPMC matrix[40], further supporting the observation that preparation of an aqueous gel prior to acetone dehydration was an important factor in more efficient polymer dehydration.

Water uptake measurement using DVS

Moisture uptake was studied in order to further understand the bioadhesion behavior of processed microparticle formulations shows the correlation between acetone volume, processing method and resultant maximum vapor sorption at 95% RH using DVS assessment.

It was observed that the sample containing the least water following processing (formulation B), demonstrated the lowest capacity for water uptake during the study. This result was unexpected as it would be supposed that the more dehydrated formulation would be more susceptible to water uptake[5,41].

Equally important are the data points for 200 ml acetone of formulations B (GAH method), E (DAM method) and H (DAH method), where the effect of processing on vapor sorption capability of the particles are striking, and the previous "water history" of HPMC appears to dictate the capability to uptake moisture. For example, direct addition of acetone to HPMC powder (formulation L, DAM method) reduces its vapor sorption potential in comparison to unprocessed HPMC powder.
hydroxypropyl group of HPMC, with an integration value of 1 as described by Gilard et al [46]. Any increase or decrease in the integration of this area indicates a change in the water content. The peak area for unprocessed HPMC was 8.104. The techniques used in this work might produce chemical changes in the HPMC instead of water content changes, so another analysis was performed at 1.1-1.2 ppm (methyl protons of the hydroxypropyl group), and was used as an indication of any chemical shift in the HPMC spectrum for processed formulations [46]. Table 4 summarizes these data.

### Table 4: NMR results of all processed formulations in comparison to HPMC

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Precipitation method</th>
<th>Number of protons at 3-5 ppm</th>
<th>Integration of peak at 1.1-1.2 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (HPMC)</td>
<td>GAH</td>
<td>8.104</td>
<td>1.143</td>
</tr>
<tr>
<td>B (gelled HPMC, 200ml acetone)</td>
<td>GAH</td>
<td>8.532</td>
<td>1.151</td>
</tr>
<tr>
<td>C (gelled HPMC, 100ml acetone)</td>
<td>GAH</td>
<td>7.748</td>
<td>1.152</td>
</tr>
<tr>
<td>D (gelled HPMC, 50ml acetone)</td>
<td>GAH</td>
<td>8.098</td>
<td>1.152</td>
</tr>
<tr>
<td>E (gelled HPMC, 200ml acetone)</td>
<td>DAM</td>
<td>8.043</td>
<td>1.145</td>
</tr>
<tr>
<td>F (gelled HPMC, 100ml acetone)</td>
<td>DAM</td>
<td>8.603</td>
<td>1.145</td>
</tr>
<tr>
<td>G (gelled HPMC, 50ml acetone)</td>
<td>DAM</td>
<td>9.613</td>
<td>1.152</td>
</tr>
<tr>
<td>H (gelled HPMC, 200ml acetone)</td>
<td>DAH</td>
<td>7.832</td>
<td>1.146</td>
</tr>
<tr>
<td>I (gelled HPMC, 100ml acetone)</td>
<td>DAH</td>
<td>8.167</td>
<td>1.145</td>
</tr>
<tr>
<td>J (gelled HPMC, 50ml acetone)</td>
<td>DAH</td>
<td>8.311</td>
<td>1.152</td>
</tr>
<tr>
<td>L (HPMC powder+ 200 ml acetone)</td>
<td>DAM</td>
<td>8.031</td>
<td>1.151</td>
</tr>
</tbody>
</table>

As can be observed in values of the peak between 1.1-1.2 ppm, no chemical changes were introduced as a result of processing HPMC. There was however changes observed in the integration values of the area between 3-5 ppm, which showed a similar trend to that discussed in the previous sections. Processing of HPMC using different precipitation techniques (GAH, DAM and DAH) produced removal of entrapped or bound water molecules (dehydration). This effect was most pronounced for the GAH technique (A, number of protons 7.532) in comparison to DAM (H, number of protons 7.832) and DAM (E, number of protons 8.043) when a fixed volume of acetone was used (200ml). As the volume of acetone decreased, the dehydration efficiency decreased and the number of protons of entrapped water molecules increased (bounded by H-bonds with HPMC) as shown in DAM formulations; E (8.043), F (8.603) and G (9.613). Direct addition of HPMC powder to acetone in formulation L produced a slight change in water content compared with untreated HPMC powder.

From these findings it was suggested that the presence or absence of water produced changes in the H-bonding of HPMC. Since the volumes of acetone used, the presence or absence of water and the forms of HPMC to be precipitated were different from one formulation to another, in addition, different precipitation techniques were used as GAH, DAM and DAH, these might cause changes in the dehydration extent and H-bonding orientation of each formulation in comparison to others.

### GENERAL DISCUSSION

Formulation B represents the exposure of a gelled HPMC to acetone, using the most efficient mixing process, and the slowest (dropwise) rate of addition of the gel to the acetone (GAH method). It would be expected therefore, that the interaction of acetone and water would be instantaneous and complete, resulting in an extremely efficient dehydration process. Surprisingly, despite this state of dehydration as evidenced by DSC, formulation B demonstrated the lowest affinity for moisture following processing as evidenced by DVS. Interestingly, this formulation also afforded the highest bioadhesive score in the mucosal surface. In the current work, it is hypothesized that the rapid dehydration of the HPMC gel resulted in a collapse of the matrix structure, forming a physical barrier to subsequent rehydration (SEM image, Figure 4a). With the other formulations and processes employed, less efficient interaction with acetone resulted in higher residual water content after processing which allowed maintenance of a more open polymer structure, thereby facilitating future moisture uptake. So the improvement in the bioadhesion of HPMC powder grade K100LV resulted from the collapsed structure, therefore having fewer groups available for hydrogenic interactions which were the cause of rapid hydration and sliding over mucin-agar adhesion medium, losing its bioadhesive property.

### CONCLUSION

A method for preparing HPMC bioadhesive microparticles for nasal route was developed using an aqueous-organic co-precipitation technique. The presence of the solvent, acetone, caused dehydration of the HPMC polymer, and the degree of dehydration depended on the volume of acetone used as a precipitating medium. As the volume of precipitating agent increased in order of 50 < 100 < 200 ml, its dehydration effect on the water molecules of HPMC aqueous gel increased. This effect was most noticeable with the dehydration of HPMC gel formulations, but was also observed to a slight extent when HPMC was treated as powder with acetone. The processing method employed for preparation of particles was also found to significantly affect the properties. The dehydration of the aqueous gel was most efficient when the gel was added dropwise to a highly agitated system. Less efficient mixing and/or lower volumes resulted in less efficient dehydration. Surprisingly, the most efficient dehydration resulted in the lowest potential for subsequent uptake of moisture, and the highest bioadhesive performance in the dynamic test employed. A hypothesis has been developed to explain the counter-intuitive behavior in response to efficiency of dehydration, residual moisture and future affinity for moisture. The most remarkable accomplishment of this work is the development of an aqueous-organic precipitation technique to formulate bioadhesive microparticles for nasal administration using low viscosity grade of HPMC (K100LV). So HPMC microparticle formulation (B) might be a respectable bioadhesive carrier for sustained, systemic and localized release of drug molecules through nasal administration.

### CONFLICT OF INTERESTS

Declared None

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### REFERENCES


