

**Characterization of Acid Treated Porcine Mucin and its Effects on Bioadhesion**

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ABSTRACT

The study aimed at determining the effect of acid modification on the physicochemical and bioadhesive properties of porcine mucin powder. Mucin powder was treated with solutions of acetic and hydrochloric acids at high and low concentrations and neutralized with equimolar volumes of pyridine and sodium hydroxide solutions, respectively after 24 h and dried. The treated powders were characterized using ultraviolet (UV) scans, solubility tests, pH, melting point, particle size analysis, Differential scanning calorimetry (DSC), Fourier transform infra-red spectroscopy (FTIR), scanning electron microscopy (SEM), powder X-ray diffractometry (PXRD) and bioadhesion measurements.

Modified powder UV scans showed a noticeable shift in their peak of absorbances, generally improved solubility, pH range of 4.20 - 4.90, melting point ranging from 105°C to 125°C and mean particle size range from 36.36 ± 6.65 to 114.25 ± 5.65 μm . Unmodified mucin DSC showed a sharp endothermic transition while those of the modified products were sharp and broad. The melting temperatures corresponded to the endothermic transitions (T_m), ranging from 105°C to 140°C. FTIR showed characteristic change in spectra pattern resulting in broadening of the OH band. PXRD showed distinct and more porous particles with the modified products. Their bioadhesion values ranged from 0.155 to 0.054 Nm^{-1} with the HCl modified mucin powders showing the highest bioadhesion.

Acid modification of native porcine mucin powders resulted in the formation of modified products with superior bioadhesive properties over the native porcine mucin powders. These modified products have a potential application in bioadhesive drug delivery systems.

Keywords: Mucin, Acid treatment, Modified, Powder, Analyses, Bioadhesion

Introduction

Polymers of various sources, whether of synthetic or natural origin continue to find increased applications in drug delivery formulations. Attempts to improve the functionality of these polymers have led to their modifications. Modified forms of some polymers have been reported to exhibit improved properties and the dosage forms formulated with them showing superior performance when compared with those of the unmodified form.^{1,2} Examples of some products that have undergone modification resulting in improved modified products includes cellulose^{1,3} and chitin.⁴ The incorporation of a sodium carboxymethyl group onto the cellulose backbone or the acid treatment of α -cellulose have resulted in sodium carboxymethyl cellulose and microcrystalline cellulose, respectively while the alkaline treatment of chitin have resulted in chitosan.⁴⁻⁶ These modifications have sometimes resulted in the total change of the polymer properties.

Mucin, a bioadhesive polymer of natural origin has been identified as useful material in drug formulations.⁷ It is highly abundant in human and animal tissues, hence it is biocompatible, non-toxic and easily

reported include thermal, pH and the effects of some chemicals on the protein moiety of the polymer.⁸⁻¹⁰ These modifications have been reported to affect mucin properties such as aggregation, gelation, stability, rheology, adhesion, crystallinity and viscosity.¹¹

This study attempts to subject porcine mucin powders to acid modification by treating the powders with a low and high concentrations of mineral and organic acid and characterizing the resulting powders in view of their physicochemical and bioadhesive properties.

Materials and Methods*Solvents and Reagents*

The following materials were purchased from local suppliers and used without further purification: porcine mucin powder (Sigma-Aldrich Chemical Company, Germany), sodium hydroxide, sodium chloride, monobasic potassium phosphate and acetone (Merck, Germany), acetic acid and dimethyl sulfoxide (DMSO) (JHD Chemicals, China), hydrochloric acid and methanol (BDH Chemicals, England), pyridine (May and Baker Ltd, England). All other reagents were of analytical grade and were used as received.

Acid modification of porcine mucin powder

Ten grams of mucin powder was weighed into each of 4 beakers. The contents of beakers 1 and 2 were mixed with 25 mL of 0.1 and 1.0 M acetic acid solution, respectively, while beakers 3 and 4 were treated with 25 mL of 0.1 and 1.0 M hydrochloric acid solution, respectively. The beakers were allowed to stand at room temperature and their contents neutralized after 24 h with equimolar volumes of sodium hydroxide solutions. The neutralized contents of the beakers were dried under vacuum at 30°C. The dried mass was pulverized into powder and stored in an airtight container until used. A schematic diagram of the modification process is shown in Figure 1.

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biodegradable. It is the polymer of choice for drug modeling of bioadhesive systems. Some modifications of mucin that have been

Characterization of mucin powders

Ultra-violet scans

The UV spectra of the modified and unmodified mucin powders were obtained by scanning the filtrate of a 1.0% w/v dispersion of the powder in distilled water over a wavelength of 200-450 nm (T70 Spectrophotometer, PG Instruments Ltd, USA) using distilled water as the blank. This was carried out to detect any alteration or differences in their spectra.

Solubility profiles

The solubility of the modified and unmodified mucin powders was determined in distilled water, 0.1 M of hydrochloric acid, sodium hydroxide, sodium chloride and DMSO at ambient temperature (28 ± 2°C). About 100 mg of powder was placed in 2 mL of solvent in a test-tube and shaken. The dispersions were allowed to stand for 24 h, filtered and the residue air dried. The dried residue and the filter paper were weighed (KERRO BL3002, England) and the solubility of the powder in the different solvents was computed from the difference in weight of the powder before and after the experiment.

pH

A 1.0% w/v dispersion of the modified and unmodified mucin powder was prepared with distilled water and allowed to stand for 1 h with the container capped at room temperature. The pH of the resultant solution was determined in triplicate using a digital pH meter (HI 2215, Hanna Instruments, USA).

Melting point

The modified and unmodified mucin powders were packed into a capillary tube sealed at one end and tapped on a hard surface for the powders to form a column at the bottom of the capillary tube. The tube was inserted into the heating block of a Gallenkamp melting point apparatus (MFB 600030 W, United Kingdom). The temperature of the heating block was raised from room temperature to the desired temperature at 1.0°C/min until the sample melted and the melting temperature was recorded.

Powder particle size

The powders of the modified and unmodified mucin samples were thinly spread over a glass slide and viewed under a light microscope (Labo Microsystems GmbH, Germany) via a calibrated eyepiece and the sizes and shapes of the particles were recorded at a magnification of ×100 (MICAM 1.4, ScopeImage 9.0).

Differential scanning calorimetry (DSC)

DSC analysis was carried out using the Netzsch DSC 204F1 Phoenix apparatus (Netzsch-Geratebau GmbH, Selb, Germany). About 1.0 mg of the sample was weighed into an aluminium pan and sealed. The seal was pierced and calibration of the calorimeter was initiated with nitrogen as the purge gas. The sample was heated at the rate of 10°C per min from 30 to 350°C under nitrogen gas at a flow rate of 70 mL/min. The analysis was carried out on the modified and unmodified mucin powder samples.

Fourier transform infra-red (FTIR) spectroscopy

The FTIR analysis of the samples was carried out using Fourier Transform Infrared Spectrophotometer (Spectrum BX, Perkin Elmer, England). The potassium bromide (KBr) pellet method was used. Five milligrams of the sample was blended with KBr to 200 mg. The powder was compressed using a Sigma KBr press into a tablet shape. The tablet was placed in the sample compartment and the IR scan was read. The modified and unmodified mucin powder samples were scanned at a range of 4000 - 750 cm⁻¹.

Scanning electron microscopy (SEM)

SEM was conducted using scanning electron microscope (EVO/MA10, Germany). Two milligrams of each powder sample was placed on the sample holder and vacuum was created using the vacuum pump. The electron gun was then aligned to finely focus the electron beam on the sample and a magnification of ×3500 was employed to examine the sample. The operating voltage was limited to 5 kV to minimize charging effect on the resolution of the images.

Powder x-ray diffractometry (PXRD)

PXRD analysis was carried out using the Shimadzu 6000 Model X-Ray Diffractometer (Shimadzu Corp., Japan). The powder was spread over a flat disc and leveled to form a plane surface. The disc with the powder was placed in the sample holder and the scan performed at a diffraction angle

of 2-theta between 2 to 60°. The patterns were recorded using NaI scintillator at 40 kV and 25 mA continuous scan for 60 min.

Bioadhesive studies

Coated beads displacement method

The determination was carried out using the method of Attama *et al.*¹². Ten dried glass beads weighing 60 mg each and average diameter of 3 mm were uniformly coated by intermittent immersion in 5.0% aqueous dispersion of the various batches of the modified and unmodified mucin and placed carefully on the surface of a freshly excised rabbit fundus of about 10 cm, pinned onto a plastic support at an angle of 30° below a separating funnel clamped to a retort stand. Ten minutes was allowed for mucus-mucin interaction at the bead-fundus interface. A 250 mL quantity of SGF without pepsin was then poured into the funnel and allowed to flow over the beads at a rate of 30 mL/min. A container was placed directly below the set-up to collect any detached beads. The number of detached beads was noted and used as a measure of bioadhesion.

Tensiometric method

This was performed using a tension balance (Model-OS, White Electrical Instrument Co Ltd, UK) adapted to measure bioadhesive strength using the Du Noüy ring method. The same concentration of mucin dispersion used in the coated bead experiment was used. A 10 mL volume of the prepared dispersions was poured into a watch glass, which was placed on the platform of the zeroed tensiometer. With the Du Noüy ring hanging on the lever arm of the tensiometer, the platform was moved up until the ring was submerged in the mucin dispersion. The tensiometer platform was moved down gradually until the ring formed a meniscus above the dispersion. The ring was raised by means of a dial until it just detached from the surface or meniscus of the mucin dispersion. The force required to raise the ring from the surface of the dispersion was recorded in Nm⁻¹. All procedures were carried out in triplicates for the modified and unmodified mucin batches.

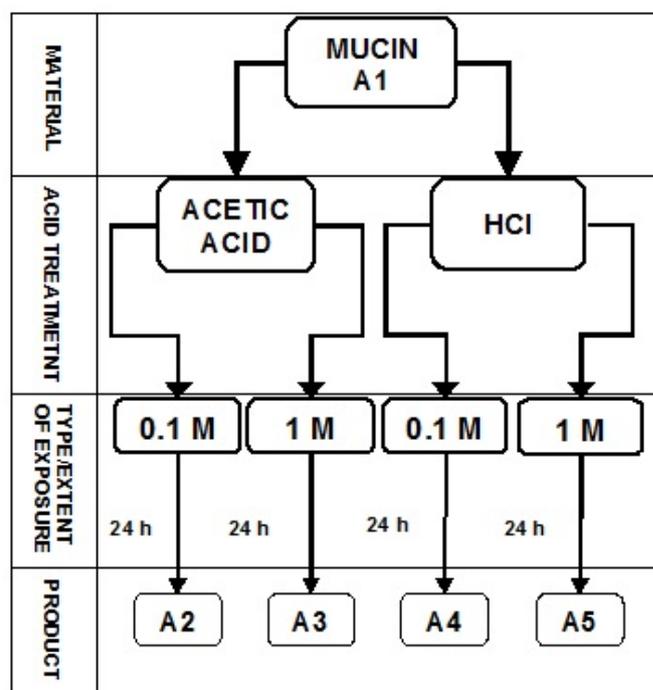


Figure 1: Schematic diagram of the acid modification process

Results and Discussion

Ultra-violet scans

The UV spectra of the modified and unmodified mucin powders are shown in Figure 2. There were sharp distinct peaks around 230 nm in A2 and A3 spectra patterns suggesting that there is some level of change occurring in the mucin powder as a result of the presence of the acetic (organic) acid irrespective of the concentration. This spike was not so distinct in A4 and A5. However, there was a narrowing of the plateau of A1 in all the acid-modified mucin ultraviolet spectra pattern. Though these differences in

spectra patterns are not conclusive. They are preliminary indications of changes in the modified products which may be as a result of some structural transformations of optically active chromophores present in the mucin molecules. Similar UV-VIS spectra differences were observed in intestinal mucin water solutions by Baranowski when subjected to basic and acidic dilutions.⁹

Properties of mucin powders

Some physicochemical parameters of the modified mucin powders are shown in Table 1. Generally, the modified products showed improved solubility at room temperature in all the test solvents except batch A5 in water. The unmodified mucin (A1) exhibited less solubility in all the test solvents with its solubility in water being the least. The solubility of the unmodified mucin agrees with previous studies on the solubility of snail mucin in water.^{7,12} The increased solubility observed in the test solvents may be as a result of the transformational changes in the mucin structure allowing more bonding between the test solvents and the modified products. These inferred changes may just be to the structural configuration of mucin, not affecting the overall structure of the glycoprotein polymer as it has been reported that a three-step acid treatment is needed to effect breakage of the molecule's glycosidic bonds.¹³

There were variations in the melting points and pH of the modified products. The variations observed were not significantly different from the unmodified mucin powder. The pH indicates that mucin is slightly acidic. That of the modified batches also showed that the acid used in the treatment was effectively neutralized. Microscopic examination of the mucin powders shows particles that are irregular in shape with a non-uniform particle size distribution. Their mean particle sizes ranged from

$36.36 \pm 6.65 \mu\text{m}$ of the A2 batches of the powder to $91.75 \pm 5.99 \mu\text{m}$ of the A5 batch. The colour of the powder samples ranged from light brown of the A1 unmodified mucin powders to the dark brown of the A4 and A5 batches.

Differential scanning calorimetry (DSC)

The DSC thermograms of the mucin powders are shown in Figure 3. The various modified products and the unmodified mucin powder showed different transitions and melting points. Their thermograms showed a characteristic endothermic transition which represents the melting points of the powders which showed a temperature close to the glass transition temperature ($125 - 135 \text{ }^\circ\text{C}$). The endothermic peak of the unmodified mucin was sharp ($105 \text{ }^\circ\text{C}$) with broad width while those of the modified product varied. Batches A2 and A3 exhibited a single sharp narrow peak suggesting their crystallinity while batches A4 and A5 gave a trough or peak over a wider temperature range of $110 - 140 \text{ }^\circ\text{C}$. These spectra pattern suggest the formation of a more specific crystalline form of the polymer in batch A3 powders with batch A2 in-between A1 and A3 indicating an incomplete transition since the acid used was dilute. Batches A4 and A5 suggest the formation of modified forms that may be more amorphous in nature than A1.

Fourier transform infra-red (FTIR)

The FTIR spectra of the modified and unmodified mucin powders are shown in Figure 4. A comparison of the spectra revealed obvious similarities with characteristic absorption spectra for -OH or -NH stretching at wavenumber of $3400 - 3550 \text{ cm}^{-1}$, and CH stretching of CH_2 and CH_3 at wavenumber of 2924 cm^{-1} . There were absorption bands between 2500 and 2100 cm^{-1} indicating CC or CN triple bond. The bands

Table 1: Some physicochemical properties of the mucin powders

Batch	Solubility				Melting Point ($^\circ\text{C}$)	pH	Mean particle size (μm) \pm SD
	H ₂ O	0.1 M DMSO	0.1 M HCl	0.1 M NaCl			
A1	+	++	++	++	122	4.20	48.75 ± 5.18
A2	+++	+++	+++	+++	120	4.68	36.36 ± 6.65
A3	+++	+++	+++	+++	115	4.56	36.92 ± 3.04
A4	+++	+++	+++	+++	115	4.60	91.10 ± 5.19
A5	++	++	+++	+++	125	4.57	91.75 ± 5.99

(+) sparingly soluble, (++) moderately soluble, (+++) soluble

Table 2: Bioadhesion of the mucin powders

Coated beads			Tensiometer	
Batch	No. of beads used	No. of detached beads	Bioadhesive strength	
			(%)	(Nm^{-1})
A1	10	5	50	0.055 ± 0.011
A2	10	5	50	0.076 ± 0.004
A3	10	4	60	0.083 ± 0.007
A4	10	2	80	0.155 ± 0.002
A5	10	2	80	0.115 ± 0.021

\pm Standard deviation

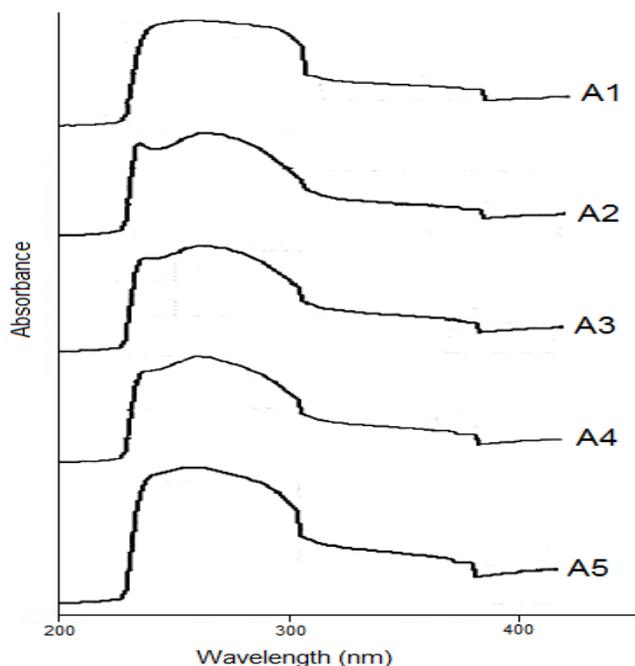


Figure 2: Ultra-violet spectra of the mucin powders A1-A5.

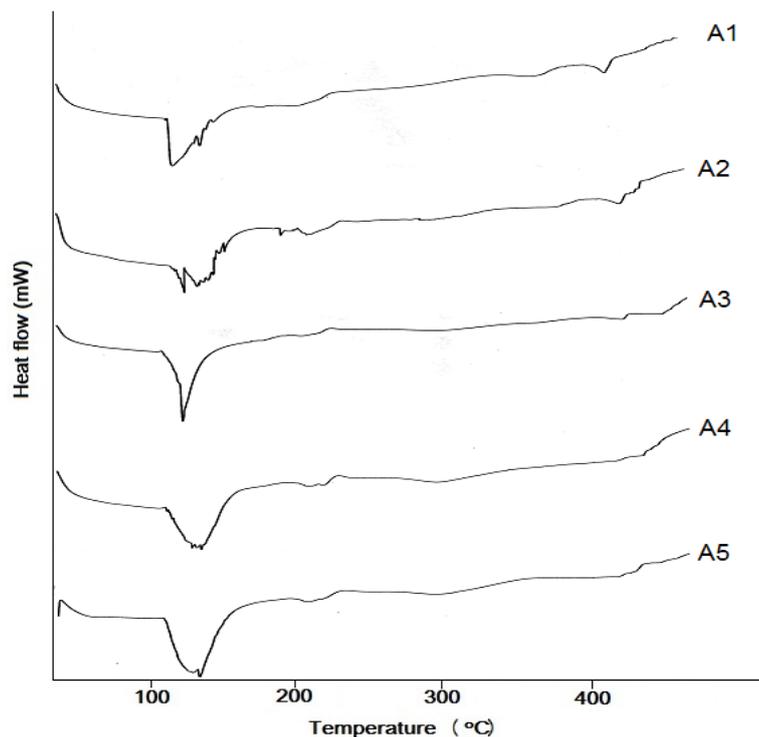


Figure 3: DSC thermograms of the mucin powders A1-A5

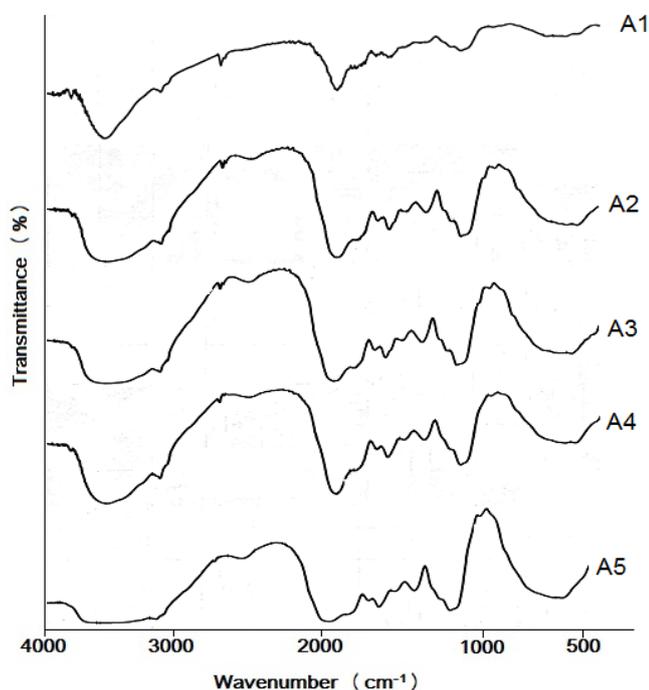


Figure 4: FTIR spectra of the mucin powders A1-A5

between 1074 and 1730 cm^{-1} are as follows: C=O stretching of acetyl or carboxylic acid (1730 cm^{-1}), H-O-H bending of absorbed water (1648 cm^{-1}), carbonyl stretching with aromatic ring (1634 cm^{-1}) and CH_2 bending (1430 cm^{-1}). The absorption bands appearing between 1000 and 1200 cm^{-1} indicate CO stretching of ether linkage (1250 cm^{-1}) C-O-C antisymmetric bridge stretching (1166 cm^{-1}), and CO symmetric stretching of primary alcohol (1062 cm^{-1}).

The basic changes shown by the spectra of the modified products are the broader peaks of the OH or NH band at wavenumber of 3400 - 3550 cm^{-1} and the more or less intensity of the various peaks at different bands. This is as a result of more hydroxylation of the modified products.¹⁴

Scanning electron microscopy (SEM)

The scanning electron micrographs of the unmodified (A1) and modified (A2-A5) mucin powders at $\times 3500$ magnification are shown in Figure 5. The results show distinct particles of spherical shape with rough and pitted surfaces of the modified powders as against the spherical shape and smooth surfaces of the unmodified mucin powder. The surfaces of the modified powders also show evidence of more pores suggesting a more porous powder compared with the unmodified powder. The major portion of the particle distribution was occupied by large particles as compared to the smaller particles which were less in number. The micrographs show tiny particles stuck on the surface of larger particles. The particle size analysis showed that size distribution varied from 40 - 50 μm for the unmodified (A1) powders as against 30 - 100 μm of the modified (A2-A5) powders. There was a clear distribution of both the small and large particles and they were not completely agglomerated. Study has shown that morphology of powders used in drug delivery plays an important role in governing the characteristics of the dosage form in which they are used¹⁵ and in this case, the wide particle size range of the modified powders may be playing a role in the improved bioadhesive strength exhibited by the modified powders.

Powder x-ray diffraction (PXRD)

The x-ray diffractograms of the modified and unmodified mucin powders are shown in Figure 6. The diffraction pattern of the modified and unmodified mucin powders exhibited similar peaks; the most prominent peaks were at $2\theta = 24^\circ$, $d = 3.689$ and of intensity 2270 counts per second for the acetic acid modified powders (A2,A3), $2\theta = 24^\circ$, $d = 3.703$ and of intensity 2966 counts per second for the hydrochloric acid modified powders (A4,A5), $2\theta = 24^\circ$, $d = 3.688$ and $2\theta = 24^\circ$, $d = 3.690$ and of intensity 1623 counts per second for the unmodified powders (A1).

Although there was no disappearance of peaks, which have been reported to imply loss or decrease in crystallinity of a material,^{16,17} the variation in intensity of the major peak reflects differences in the acid strength used in the treatment of the powder.¹⁸ Similar increases in intensity were exhibited in the smaller peaks of the modified powders when compared with the unmodified powder. These increases in intensities of peaks could have resulted from a higher crystallinity of the modified powders.

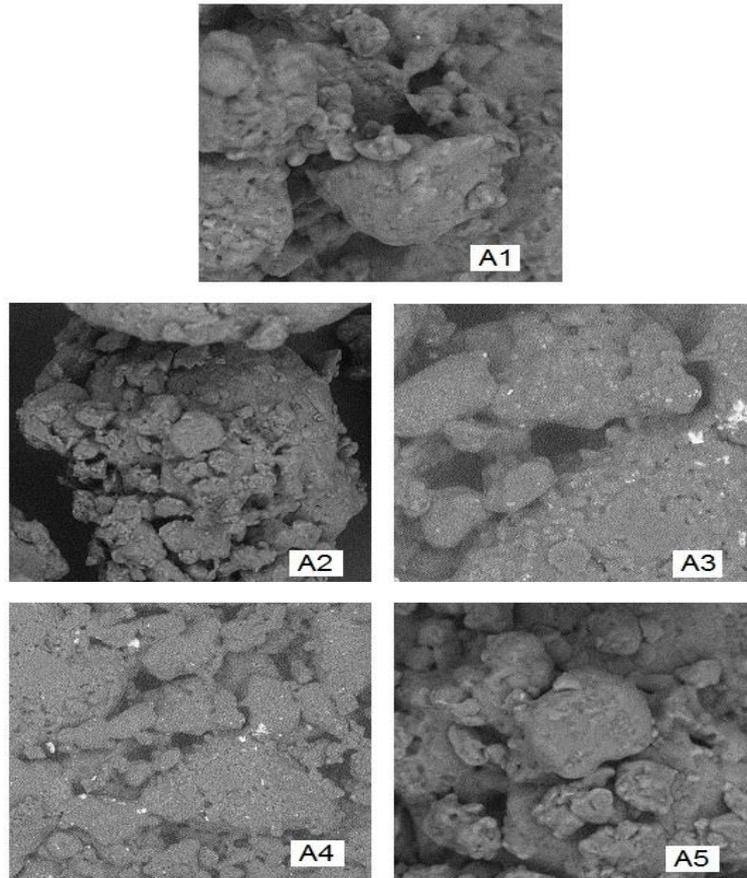


Figure 5: Scanning electron micrographs of the mucin powders A1-A5

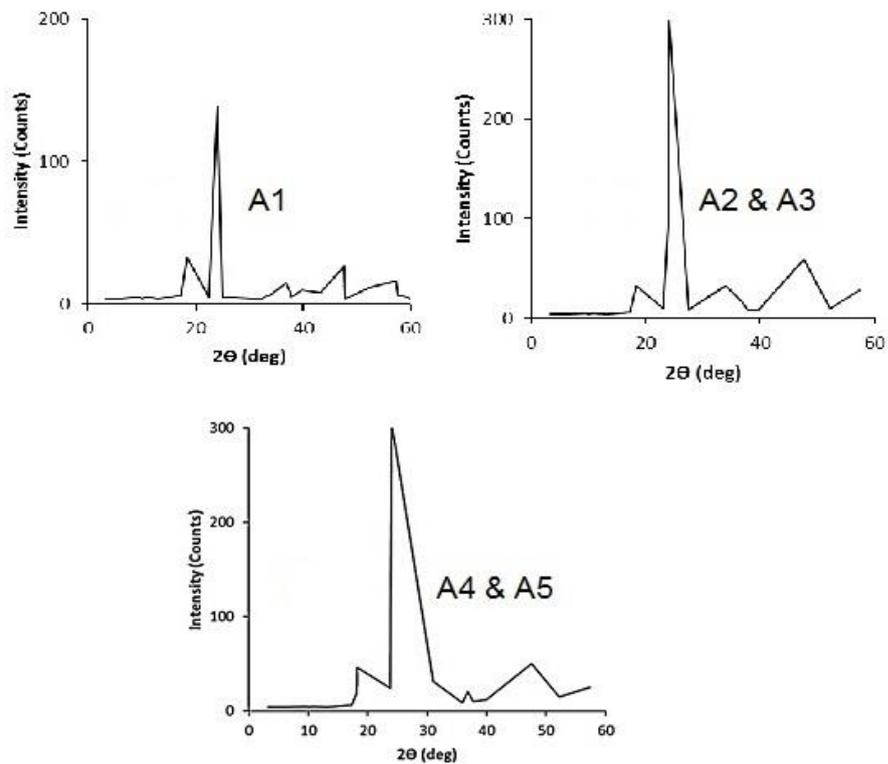


Figure 6: PXRD diffractograms of the mucin powders A1-A5.

Bioadhesion

Results from the bioadhesion studies using coated beads displacement method are shown in Table 2. The results indicate a 50 - 60 % bioadhesion for the acetic acid modified (A2, A3) mucin powders as against the 80 % bioadhesion of the hydrochloric acid modified (A4, A5) mucin powders. The unmodified mucin powder gave a bioadhesion of 50 %. Although the coated beads test for bioadhesion is very simple and easy to carry out, it is also highly subjective. Despite its limitation, it gave insight into the relative bioadhesiveness of different modified and unmodified mucin powders. Bioadhesion determination using this method is dependent on the mucus content of the rabbit intestinal surface, speed of washing and angle of inclination of the surface on which the rabbit fundus is placed. The tensiometer results (Table 2) show bioadhesion values ranging from 0.054 - 0.155 Nm⁻¹. The hydrochloric acid modified batches of mucin powders gave the highest bioadhesion values followed by the acetic acid modified batches. This result also conforms to that obtained using the coated beads displacement method. The increase in bioadhesive strength from the unmodified mucin powder through the acetic acid modified batches to the hydrochloric acid modified batches of powder would suggest a modification process or changes in the powder resulting in increased bioadhesion and with this increase depending on acid strength. Acetic acid as a weak organic acid produced a non-significant change while the stronger inorganic hydrochloric acid produced a more significant change in the mucin powders ($p < 0.05$). However, it may appear that a higher concentration of the inorganic acid may not necessarily result in changes favouring increased bioadhesion as seen in the reduction of bioadhesive strength of the A5 powders.

Conclusion

Acid modification of native porcine mucin powders resulted in the formation of modified products which were more soluble in the tested solvents and they also showed a reduction in their melting points. The modified powders were also more porous and crystalline with superior bioadhesive strengths. These properties make the modified products superior to the native porcine mucin powders in their applications in bioadhesive drug delivery systems.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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