www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2016; 5(12): 08-12

www.thepharmajournal.com
Received: 03-10-2016
Accepted: 04-11-2016

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An investigation of the properties of mucin obtained from three sources

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Abstract
The purpose of this study was to carry out a comparative evaluation of the physicochemical properties of mucin powders extracted from three different sources.

Mucin powders was extracted from the African giant snails and the intestines of cow and pigs by the wet rendering process and differential precipitation with chilled acetone. The precipitates were air-dried and pulverized into powder. The powders were subjected to different organoleptic and physicochemical evaluations including solubility profiles, pH, moisture content and particle size. Their powder flow properties such as bulk and tapped densities, true density, angle of repose, flow rate, Hausner's ratio and Carr's compressibility index were also evaluated.

The mucin powders showed comparable organoleptic properties, solubility profiles, melting points and pH. The powders were positive to tests for carbohydrates and proteins with traces of fixed oil. Microscopic examination of their particles reveals particle size and size distribution from 60 - 88 µm. There were slight variations in the bulk properties of the powders which exhibited good to fair flowability with the following parameters; Hausner's ratio (1.11 - 1.30), Carr's index (9.99 - 18.44%), angles of repose (38.26 - 40.02°) and flow rate (2.68 - 3.25 g/sec). Their moisture content ranged from 10 - 16%.

Results of the study has shown that the snail, bovine and porcine mucin powders are comparable in quality and thus may be considered equivalent when being used as an adjuvant in mucoadhesive drug delivery systems.

Keywords: Snail, bovine, porcine, physicochemical properties, mucin, mucoadhesives

1. Introduction
Mucoadhesives are synthetic or natural polymers that interact with the mucus layer covering the mucosal epithelial surface and they are the main molecules constituting a major part of mucus [1]. The concept of mucoadhesives has attracted the interest of many investigators and the possibility that these polymers can be used to overcome physiological barriers in long-term drug delivery is now receiving significant attention.

Extensive research efforts throughout the world have resulted in significant advances in understanding the various aspects of mucoadhesion. The research on mucoadhesives, however, is still in its early stage, and further advances need to be made for the successful translation of the concept into practical application in controlled drug delivery [2].

A number of mucoadhesive-based dosage forms, including sustained release tablets, semisolid forms, powders, and micro- and/or nanoparticles for use in the gastro-intestinal tract, nasal tract, cornea, buccal cavity, vagina and rectum have been widely studied [3-6].

Mucin, a glycoprotein with mucoadhesive properties is ubiquitous in many human and animal tissues. It can be found in the intestine, eye, ovaries, salivary glands and many other tissues and organs in the body. Its negative charge makes it a good candidate for drug delivery as it can be conjugated to positively charged drug molecules and targeted to various tissues. It is highly biocompatible, non-toxic and easily biodegradable, and it is often used for drug modelling of bioadhesive systems [7, 8]. The interaction of various polymers at the mucin-polymer interface is often used to explain the mechanism of mucoadhesion. The molecular bridges which result between mucin-polymer interaction account for the adhesive strength. Apart from these bridges, the electronic properties of mucin help in mucoadhesion. The use of the mucoadhesive properties of mucin have been extensively studied [9-11]. Its high potentials as a pharmaceutical excipients has not been fully explored.

While several studies have been carried out with mucin from various sources, this study was designed to compare the properties of mucin extracted from the African giant snail and the intestines of cow and pig that could influence the performance of dosage forms.

2. Materials and methods

2.1 Materials

The following chemicals were purchased from their suppliers and used without further purification. Acetone (Merck, Germany), ethanol and hydrochloric acid (BDH Chemicals, England), sodium hydroxide and ammonium hydroxide (JHD Chemicals, China). Terrestrial African giant snails were purchased from a local market while the small intestines of freshly slaughtered cows and pigs were obtained from a government approved abattoir in Benin City, Edo State, Nigeria. All other chemicals used were reagent grade.

2.2 Extraction of mucin

Snail mucin powder was extracted from the African giant snail *Archachatina marginata* following the method of Adikwu [12]. The snail shells were cracked and their fleshy bodies removed from the shells with the aid of a metal rod. Excretory materials accompanying the bodies were removed. A total weight of 10 g of the snail bodies was subjected to washing by squeezing off the slime from the fleshy bodies repeatedly into a pool of 250 ml of water and decanted. This procedure was repeated 2 more times to give a total decanted pool of 1 L. Mucin was precipitated out of the pooled washings using 2 L of chilled acetone. The precipitate was filtered and lyophilized to give brownish flakes. The dried flakes were blended in an electric blender to give mucin powders.

The bovine and porcine mucin were extracted using the method of Ofokansi and Adikwu [13] with some modifications. The cow and pig intestines were dissected, starting from the jejunum to the ileocaecal sphincter. The intestines were sectioned into short lengths and flushed through with chilled saline. The mucosal surfaces of the intestines were exposed by longitudinal dissection. Using a glass slide, the mucus layer was gently scraped off and diluted to four times its volume with distilled water to give 1 L. The gel mixture formed was homogenized and precipitation was carried out with 2 L of chilled acetone. The precipitate was filtered and lyophilized to give flakes. The dried flakes were blended in an electric blender to obtain the bovine and porcine mucin powders. The powders were stored in an airtight container until use.

2.3 Characterization of mucin powder

2.3.1 Organoleptic properties

The taste, odour, colour and texture of the mucin powders were tested by five different individuals and a score sheet was assigned to which each assessor indicated their respective impression. The average score was computed.

2.3.2 Solubility profile

The solubility of 100 mg mucin powder in 2 ml of the test liquid in a test-tube was determined at ambient temperature (25 °C), 30 and 35 °C. The powder dispersion was raised to the required temperatures in a thermostated water bath with intermittent shaking and filtered with a pre-weighed filter paper. The residue was air dried and the filter paper with the residue was weighed using a sensitive balance (KERRO BL3002, England) and the difference in weight was used as a measure of solubility of the mucin powders.

2.3.3 Melting point

The mucin powders was 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. The temperature of the heating block was raised from room temperature at 0.5 °C per min until the sample melted and the melting temperature was recorded. Triplicate determinations were carried out and the average melting temperature computed.

2.3.4 pH determination

A 1%w/v dispersion of the 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 (Hanna Instruments, USA).

2.4 Chemical tests

2.4.1 Test for Carbohydrates

Fehling's test: Freshly prepared Fehling's solution A and B were added to 1 ml of a 1%w/v aqueous dispersion of the mucin powders and heated in a water bath for 5 min. The resulting colour change was recorded.

Molisch's test: Two drops of alpha-naphthol solution was added to 2 ml of 1%w/v aqueous dispersion of the mucin powder in a test tube and 1 ml of concentrated sulphuric acid was carefully poured down the side of the test tube.

2.4.1.1 Tollen's test: Two milliliters of 1%w/v aqueous dispersion of the mucin powder in a test tube was treated with 2 ml of Tollen's reagent. The test tube was placed in a boiling water bath for about 10 min and the colour of the precipitate formed was recorded.

2.4.2 Test for proteins

Biuret test: About 10 mg of the mucin powder was placed in a test tube and moistened with a few drop of water and 1 ml of dilute sodium hydroxide solution. Copper solution (1%) was added drop-wise and the dispersion shaken with each drop until an observable colour change.

2.4.2.1 Millon test: A 5 ml quantity of Millon's reagent was added to a 2 ml dispersion of the mucin powder in a test tube and heated for 5 min. The dispersion colour change was noted.

2.4.2.2 Xanthoproteic reaction: A 5 ml dispersion of the mucin powder in a test tube was added a few drops of concentrated nitric acid. A white precipitate was formed which turns yellow on heating. The content of the test tube was cooled and few drops of ammonia solution was added and the colour of the precipitate was recorded.

2.4.3 Test for fixed oils

A drop of a 10%w/v acetone dispersion of the mucin powder was place on a filter paper. The filter paper was air-dried and the drop spot examined for translucency.

2.5 Microscopy

The mucin powder samples were thinly spread over a glass slide and viewed under a microscope (Labo Microsystems GmbH, Germany) via a calibrated eye piece. The sizes and shapes of the mucin powder particles were measured at a magnification of ×40 (MICAM 1.4, Scope Image 9.0).

2.6 Bulk density

Mucin powder (20 g) was weighed and poured gently into a 100 ml measuring cylinder. The volume occupied by the powder was recorded as the bulk volume. Triplicate determinations was carried out and the average value generated was used to calculate the bulk density employing Eq. (1).

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Volume of powder}} \ldots\ldots \quad (1)$$

2.7 Tapped density

The measuring cylinder containing the 20 g powder was tapped mechanically on a flat surface for about a 100 times to a constant volume which was recorded as the tapped volume. Triplicate determinations were carried out and the average value generated were used to calculate the tapped density using Eq. (2).

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Tapped volume of powder}} \ldots\ldots.. \quad (2)$$

2.8 Carr's (Compressibility) index

Using Eq. (3), the difference between the tapped and bulk density of the mucin powders divided by the tapped density was calculated and the ratio expressed as percentage to give the Carr's index.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \cdots. \quad (3)$$

2.9 Hausner's ratio

The ratio of the tapped density to the bulk density of the mucin powders was calculated as the Hausner's ratio or quotient with Eq. (4).

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \ldots\ldots \quad (4)$$

2.10 True (particle) density

A 25 ml specific gravity bottle (glass pycnometer) was filled with liquid paraffin, cleaned of any residual liquid paraffin and weighed (a). The bottle was emptied, rinsed with acetone and dried. About 1 g (b) of the mucin powder was poured into the bottle and then filled with liquid paraffin. It was weighed (c) after cleaning off the residual paraffin from the bottle. The various weights recorded were used to calculate the true density of the mucin powder using Eq. (5) [14-16]. The tests were carried out for all the mucin powders in triplicates.

$$\text{Particle density} = \frac{b}{[(a+b)-c]S} \quad \ldots\ldots \quad (5)$$

Where S is the specific gravity of liquid paraffin

2.11 Flow rate

The time taken for 20 g of the mucin powder to pass through the orifice of an Erweka flow tester was recorded. This was carried out in triplicates and the mean values recorded.

2.12 Angle of repose

The hollow tube method was used. A short hollow tube of 3 cm in internal diameter sitting on a circular horizontal surface of same diameter was filled with mucin powder. The tube was withdrawn vertically and excess powders allowed to fall off the edge of the circular horizontal surface. The height of the heap was measured. The angle of repose, θ, was calculated using Eq. (6).

$$\theta = \tan^{-1}\frac{h}{r} \ldots\ldots \quad (6)$$

Where h is the height of the heap of powder and r is the radius of the circular base

2.13 Moisture content

A 1 g quantity of the mucin powder was dried on a pre-weighed filter paper in a hot air oven for 30 min at 45 ºC. The initial weight of the powder and the weight after drying were recorded and used to calculate the moisture content.

2.14 DSC characterization of the mucin powders

DSC characterization of the mucin powders was carried out using the Netzsch DSC 204F1 Phoenix apparatus (Netzsch Germany). Four milligrams of the sample was weighed into an aluminium pan. The seal was pierced and calibration of the calorimeter was done with indium and the purge gas was nitrogen. Heating of the sample was carried out at the rate of 10 °C per min from 30 to 350 °C under nitrogen at a flow rate of 70 ml/min

2.15 Statistical analysis

Data obtained were subjected to the student's t-test at 5% level of significance using GraphPad In Stat 3.10.

3. Results and discussions

3.1 Organoleptic properties

The organoleptic properties of the mucin powders are shown in Table 1. The mucin powders were brownish in appearance with the bovine and porcine mucin being of a lighter shade. The powders were tasteless but the snail mucin was characteristic in odour while that of bovine and porcine mucins had a pleasant meaty odour. All the mucin powders had a powdery smooth texture.

Table 1: Organoleptic properties of the mucin powders

Properties	Mucin		
	Snail	Bovine	Porcine
Appearance	Brown	Light brown	Light brown
Taste	Tasteless	Tasteless	Tasteless
Odour	Characteristic	Pleasant meaty	Pleasant meaty
Texture	Powdery fine	Powdery fine	Powdery fine

3.2 Solubility profile, pH, melting point and chemical tests

Some physicochemical parameters of the mucin powders are shown in Table 2. Generally, the powders showed increased solubility in all the test solvents with increase in temperature. The mucin powders exhibited partial solubility in distilled water and 0.1 M sodium hydroxide solution and complete solubility in 0.1 M dimethyl sulfoxide (DMSO). All the other solvent did not show any level of solubility at all even with increase in temperature. The solubility profiles of the mucin powders agrees with previous studies on the solubility of snail mucin in water [9, 17]. The increased solubility observed with increase in temperature 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 mucin molecule. The melting points of the mucin powder was between 115 - 120 °C. The pHs of all the mucin powders were slightly acidic. Results from the chemical tests on mucin powders are shown in Table 3. The powders were rich in carbohydrates and proteins with some trace amounts of fixed oil. The results confirms the fact that mucin is a glycoprotein. Similarity in the tests results also confirms similarity in their constituent sugar and amino acid moieties.

Table 2: Solubility profiles, melting point and pH of the mucin powders

Mucin		Solubility						Melting Point (ºC)	pH
	Temp (ºC)	H_2O	Acetone	Ethanol	NaOH (0.1 M)	HCl (0.1 M)	DMSO (0.1 M)		
Snail	25	-	-	-	-	-	+	120	6.70
	30	+	-	-	-	-	++		
	35	+	-	-	++	-	+++		
Bovine	25	-	-	-	-	-	+	120	5.80
	30	-	-	-	-	-	+++		
	35	+	-	-	++	-	+++		
Porcine	25	-	-	-	-	-	++	115	5.20
	30	+	-	-	-	-	+++		
	35	+	-	-	++	-	+++		

(+) sparingly soluble, (++) moderately soluble, (-) not soluble

Table 3: Some chemical properties of the mucin powders

Tests	Mucin		
	Snail	Bovine	Porcine
Test for carbohydrate			
Fehling	++	++	+++
Molisch	++	+++	++
Tollen	+++	++	++
Test for protein			
Biuret	++	++	++
Millon	++	++	++
Xanthoproteic	++	++	++
Test for fixed oil	+	+	+

(+) trace amounts, (++) moderate amounts, (+++) copious amounts

Table 4: Some physical properties of the mucin powders

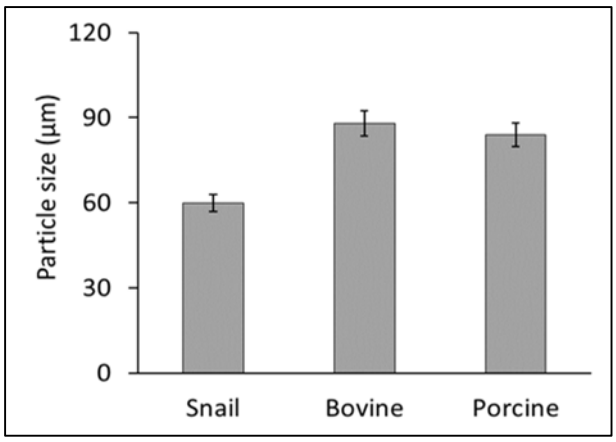
Powder properties	Mucin		
	Snail	Bovine	Porcine
Bulk density (g/cm^3)	0.671 ± 0.021	0.604 ± 0.011	0.644 ± 0.012
Tapped density (g/cm^3)	0.746 ± 0.104	0.789 ± 0.035	0.748 ± 0.033
Hausner's ratio	1.11 ± 0.22	1.37 ± 0.14	1.30 ± 0.18
Carr's index (%)	9.99 ± 1.25	16.80 ± 1.12	18.44 ± 1.82
True density (g/cm^3)	0.15 ± 0.01	0.12 ± 0.08	0.13 ± 0.02
Flow rate (g/sec)	2.68 ± 0.55	3.20 ± 0.46	3.25 ± 0.54
Angle of repose (°)	38.26 ± 1.02	40.02 ± 1.13	39.85 ± 1.10
Moisture content (%)	10.05 ± 2.54	16.10 ± 1.20	14.90 ± 1.84

Mean ± standard deviation

3.3 Physical properties

Results from the microscopic examination of the mucin powder particles showed a range of sizes from 60 - 88 µm (Fig. 1) with a narrow size range. The mucin powders particles were mostly spherical or oval or pear shaped. There was no significant ($p > 0.05$) difference amongst the mucin powders with regards to their particle sizes and this could have been influenced by the size reduction of the milling process.

The mucin powders bulk and tapped densities are shown in Table 4. These values shows a higher volume reduction for the bovine mucin powders, followed by the porcine and then the snail mucin powder. The implication is that the snail mucin powders have a higher powder consolidation (close packing) which could be as a result of its particle size and size distribution while on the other hand, the larger particle size and size distribution of the bovine and porcine mucin powders would facilitate the smaller particles filling the void spaces created by larger ones. This is in line with Newman, [18] who showed that low densities of powders result when void spaces created by larger particles are filled by smaller particles, leading to consolidation of the powder.



Hausner's ratio and Carr's index are indirect methods of assessing the flow properties of powders. For Hausner's ratio, values greater than 1.6 are indicative of poor flowability while values greater than or equal to 1.25 show good flowability. Carr's index values less than or equal to 16% indicates good flowability while values greater than 23% demonstrate poor flowability [19, 20]. The result shown in Table 4 indicate that all the flowability of the mucin powders is of the order; snail > porcine > bovine. The angle of repose is also another indirect method of accessing the flow properties of powders. As a general guide, powders with angle of repose greater than 50° have unsatisfactory flow properties, whereas minimum angles close to 25° correspond to very good flow properties [20]. The results obtained for all the batches where above 25° but below 50°. Therefore, all the mucin powders can be said to have fair flow properties in the order of porcine > bovine > snail.

The moisture content of the mucin powders ranged from 10 - 16% with the bovine mucin powders having the highest value. The varied moisture content of the powders may be as a result of the larger particle size of the bovine mucin powders leading to larger pore sizes which may trap water and result in high moisture content [21].

4. Conclusion

This study has shown that the test mucin powders, locally sourced and extracted from the African giant snail and the intestines of cow and pig are comparable in quality and thus may be considered equivalent when being used as an adjuvant in mucoadhesive drug delivery systems.

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