Characterization of Grewia Gum, a Potential Pharmaceutical Excipient

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ABSTRACT

Grewia gum was extracted from the inner stem bark of *Grewia mollis* and characterized by several techniques such as gas chromatography (GC), gel permeation chromatography (GPC), scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and thermogravimetric analysis of the extracted sample. Spectroscopic techniques such as x-ray photoelectron spectroscopy (XPS), fourier-transformed infrared (FT-IR), solid-state nuclear magnetic resonance (NMR), and ^{1}H and ^{13}C NMR techniques were also used to characterize the gum. The results showed that grewia gum is a typically amorphous polysaccharide gum containing glucose, rhamnose, galactose, arabinose and xylose as neutral sugars. It has an average molecular weight of 5925 kDa expressed as the pullulan equivalent. The gum slowly hydrated in water, dispersing and swelling to form a highly viscous dispersion exhibiting pseudoplastic flow behaviour. The polysaccharide gum is thermally stable and may have application as stabilizer or suspending agent in foods, cosmetics and in pharmaceuticals. It may have application as a binder or sustained-release polymer matrix in tablets or granulations.

KEY WORDS: grewia gum, extraction, polysaccharide, excipient, suspending agent, binder, sustained release

INTRODUCTION

Natural polysaccharide gums represent a group of polymers which swell to form highly viscous solutions or dispersions in aqueous media. They have found wide application in pharmaceutical formulations such as polymer matrices in sustained release solid dosage forms (1-5), binders in tablets (6), stabilizers or suspending agents in liquid dosage forms (7), and in bioadhesive drug delivery systems (8).

Polysaccharide gums used in the pharmaceutical and food industries include guar

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gum, tragacanth, acacia gum or gum Arabic and xanthan gum amongst others. They have the advantage of biocompatibility, low cost and relatively wide spread availability compared to their synthetic counterparts (9).

Grewia polysaccharide gum is obtained by extraction from the inner stem bark of the edible plant *Grewia mollis,* Juss, (Fam. Tiliaceae). In Nigeria, grewia polysaccharide gum grows abundantly in the middle belt region of the country where it is found growing wild or cultivated and is used as a food delicacy by the local people. The gum has been isolated and some of its physicochemical properties have been evaluated (10). The polysaccharide gum consists of glucose and rhamnose as the main monosaccharide components and galacturonic acid as the main sugar acid (10). The binding (11), bioadhesive (12) and mechanical (13) properties of the gum has been reported. Grewia polysaccharide gum may provide an alternative to other natural polysaccharide gums or their synthetic counterparts, and save foreign exchange in the regions of the world where it is in abundant supply.

The extraction and characterization of polysaccharide gums is an essential step in establishing their suitability as pharmaceutical excipients. In this work, the physicochemical properties of the gum were evaluated including viscosity and flow characteristics, elemental composition and surface chemistry, molecular weight and thermal properties. Compositional analysis of the gum was carried out and spectroscopic techniques were also used to analyse the material.

MATERIALS AND METHODS

Materials

Ethanol, sodium hydroxide and hydrochloric acid were purchased from Sigma Chemical Co. (Dorset, UK), pullulan polysaccharides from Polymer Laboratories (UK), and pulverized crude inner stem bark of *Grewia mollis* shrub was obtained from Nigeria after authentication by the forestry division. All equipment used is cited in the text.

Extraction and purification of grewia gum

The dried and pulverized inner stem bark of *Grewia mollis* was dispersed in 0.1% w/v sodium metabisulphite solution and allowed to hydrate for 48 hours. After this time the mixture was stirred for 2 hours and passed through a muslin bag to remove extraneous materials. The filtrate was treated with 0.1N NaOH and centrifuged at 3,000 rpm for 10 minutes. The supernatant was then treated with acidified ethanol, containing 0.1N HCl, and centrifuged again as described previously. The supernatant was treated with absolute ethanol and the resultant precipitate washed several times until only clear ethanol was recovered. The precipitate was wet-milled and then filtered through muslin to remove excess ethanol before air-drying the product. Thereupon the air-dried product was dry-milled before further drying at 50°C in an oven for 24 hours. The dried product was passed through a 1.0 mm sieve, weighed and stored in air-tight containers.

Total ash and soluble ash

The total and soluble ash was determined according to the AOAC method (14). A 1.0 gram portion of the gum was weighed into a pre-ignited and pre-weighed crucible, and transferred into a furnace (Carbolite, Sheffield-England). The ignition temperature was 550°C for 24 hours. The recovered ash was transferred into a desiccator to equilibrate to room temperature before weighing. In order to determine soluble ash, the resultant ash from above was mixed with distilled water (25 ml), boiled and filtered through ash-less filter paper. The filter paper was then rinsed until the filtrate volume reached 60 ml. Both filter paper and residue were transferred to the crucible and ignited for 24 hours until a constant weight was

reached. Thereafter, it was cooled in a desiccator and weighed.

Percent total ash was calculated from the formula:

$$\% \text{ Total ash} = \frac{\text{Ash weight}}{\text{Original sample weight}} \times 100 \quad \text{Eq 1}$$

Percent soluble ash was calculated from the formula:

$$\% \text{ Soluble ash} = \% \text{ Total ash} - \% \text{ Insoluble ash} \quad \text{Eq 2}$$

Aqueous solubility

The aqueous solubility of grewia gum was determined gravimetrically by accurately weighing 1g into 1000 ml of distilled water and hydrated at room temperature for 24 hours. Thereupon, the dispersion was filtered through a pre-weighed filter paper of medium porosity. The residue on the filter paper was weighed after drying in an oven at 50°C for 24 hours and the difference determined.

Viscosity and flow behaviour

The viscosity of a 1.0% w/v dispersion of the polysaccharide gum was read at shear rates between 0.1 to 2.0 reciprocal seconds and at 23°C using a Brookfield DV – 1+ viscometer version 5 (Brookfield Engineering Labs, Stoughton-USA). Spindle 2 was used and 3 minutes was allowed for stabilization of the readings before the viscosity was read.

Effect of temperature and electrolytes on viscosity

The viscosity of a 0.5% w/v dispersion of the gum was determined at 10°, 20°, 30°, and 40°C using a viscometer as above at 0.1 reciprocal seconds using spindle 2. The effect of electrolyte type and concentration on viscosity of the gum was determined by gradually increasing the concentration of electrolyte in a 0.5% dispersion of the gum from 0.125 to 1.0 molar solutions using KCl, $CaCl_2$ or $AlCl_3$.

Angle of repose

Grewia polysaccharide gum powder (10 g) was accurately weighed and carefully introduced into a funnel clamped to a stand with its tip 10 cm from a plane paper surface. The powder was allowed to flow freely unto the paper surface. The height of the cone, *H* formed after complete flow and the radius of the cone, *R* were measured and used to calculate the angle of repose using the equation:

$$\text{Angle of repose } (\tan\theta) = H/R \quad \text{Eq 3}$$

Bulk and tapped densities

The polysaccharide gum powder (10 g) was accurately weighed into a 100 ml measuring cylinder and without disturbing the cylinder the volume of powder was read to give the bulk volume. The measuring cylinder was then clamped to the USP I tapper of a USP tap density tester (Sotax TD2, Switzerland).The volume of the powder was read after every 50 taps up to a total of 300 taps when volume of powder was constant. This represents the tapped volume of the powder. The bulk density and tapped density was calculated using equation 4 and 5 respectively.

$$\text{Tapped density } (\rho_t) = \frac{\text{weight of sample}}{\text{tapped volume}} \quad \text{Eq 4}$$

$$\text{Bulk density } (\rho_b) = \frac{\text{weight of sample}}{\text{bulk volume}} \quad \text{Eq 5}$$

Hausner quotient

Hausner ratio, quotient or factor was calculated as the ratio of tapped to bulk densities (equation 6).

$$\text{Hausner's quotient (ratio)} = \frac{\text{tapped density}}{\text{bulk density}} \qquad \text{Eq 6}$$

Compressibility index

The compressibility index of the polysaccharide gum was determined according to the Carr's compressibility index percentage (see equation 7).

$$\text{Carr's compressibility} = \frac{\text{tapped density - bulk density}}{\text{tapped density}} x100 \qquad \text{Eq 7}$$

Scanning electron microscopy

Scanning electron micrograph of the grewia gum sample was carried out (StereoScan 90, Cambridge instruments) after gold coating using an Enscope SC500 gold sputter coater.

Hydrolysis, monosaccharide sugars analysis and ^{1}H and ^{13}C NMR

Before GC and NMR analysis of the sample, the gum was hydrolysed according to the method of Tamoda *et al*., (15). The hydrolysed gum and sugar standards were converted to the alditol acetate derivatives and thereupon monosaccharide analysis was done on an ATI – Unicam 610 GC, (Unicam, UK) with a flame ionization detector (FID). The conditions were: Column- Supelco SP-2380 (30 m, 0.25 mm id and 0.2 μm film thickness), detector temperature- 280°C, injector temperature- 260°C, column temperature- 250°C, split injection volume- 1 μL, split ratio- 50:1, Helium flow- 1 ml/minute, and 15 minute isothermal run. The standard sugars used were L-rhamnose monohydrate, D-(+)-galactose, DL-arabinose D-xylose and D-(+)-glucose. ^{13}C and ^{1}H NMR spectroscopy was done on an AC 250 NMR spectrometer (Bruker, UK) in D_2O and analysed using Iconnmr and Topspin (Bruker) software.

FT-IR Spectroscopy

KBr discs, obtained by blending and compressing a small amount of the gum in KBr (1:10) on an IR press, were scanned on a Mattson Galaxy 3020 FT-IR spectrophotometer (Unicam, England).

Solid-state NMR

A Varian VNMRS spectrometer operating at 102.56 MHz for ^{13}C was used for the analysis of the gum samples. The samples were run without modification and the spectra were referenced with respect to tetramethylsilane.

X-ray Photoelectron spectroscopy

The analysis was conducted in a Thermofisher ESCALAB 250 electron spectrometer equipped with a hemispherical sector energy analyzer. Monochromatic Al K_{α} X-ray source was used at a source excitation energy of 15 KeV and emission current of 6 mA. Analyzer path energy of 20 eV with step size of 0.1 eV and dwell time of 50 ms was used throughout the experiments.

Thermal analysis

A Thermo-gravimetric analyzer (Pyris 1 TGA Perkin Elmer, U.S.A) was used to study the thermal degradation of the polysaccharide gum under nitrogen atmosphere. Approximately 1 mg of sample was introduced into the sample pan and heated at 10°C per minute up to 500°C. Results were obtained in triplicate and the representative plots and derivatives collated. Differential Scanning Calorimetry (Diamond DSC Perkin Elmer, USA) was also used to study the thermal properties of the grewia polysaccharide gum. About 4 mg of sample was accurately weighed into the sample pan and the temperature was held at 30°C for 1 minute before heating up to 200°C at a rate of 10°C per minute under a nitrogen atmosphere.

Gel permeation chromatography

Gel permeation chromatography was carried out to estimate molecular weight of the gum relative to pullulan polysaccharide calibrants. The eluent used was 0.2M $NaNO_3$ and 0.01M NaH_2PO_4 (pH of 7.0) at a nominal flow rate of

NaH_2PO_4 (pH of 7.0) at a nominal flow rate of 1.0 ml per minute and a temperature of 35°C. The columns used were Plaquagel Guard Plus 2 x mixed-OH, 30 cm 8μm columns and a refractive index detector (with differential pressure and light scattering) was used. The data were collated and analysed using Polymer Laboratories 'Cirrus' software. The sample was prepared by adding 10 ml of eluent to 20 mg of sample, hydrating overnight and warming to 40°C for at least 20 minutes. After cooling, the solutions were thoroughly mixed and filtered through a 0.45 μm PVDF membrane prior to injection on the column.

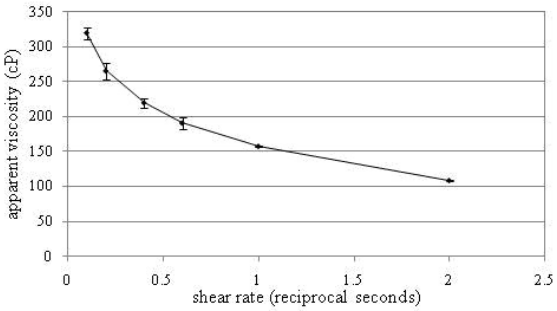

Figure 1 Pseudoplastic flow (shear-thinning) behaviour of a 1% w/w aqueous dispersion of grewia gum at room temperature (n=3, mean ± s.d.).

RESULTS AND DISCUSSION

Physicochemical properties of grewia gum

The yield of the gum after extraction from the crude inner stem bark of the plant was 32.4%. Physicochemical properties of the gum are summarised in Table 1. Grewia polysaccharide gum Grewia polysaccharide gum slowly hydrates and swells in water. The gum has aqueous solubility of about 0.2 mg/ml. The low solubility of the gum may be attributable to insoluble cell-wall materials making up a larger proportion of the gum.

Table 1 Physicochemical properties of grewia gum

Solubility (*mg/ml*)	0.2 ± 0.01
Soluble ash (%)	3.4 ± 0.20
Total ash (%)	6.1 ± 0.36
pH	5.7 ± 0.03
Moisture content (%)	10.6 ± 2.01
Viscosity at 0.1 Hertz (*cP*)	319.1 ± 8.68
Angle of repose (°)	30.4 ± 0.47
Bulk density (*g/ml*)	0.16 ± 0.00
Tapped density (*g/ml*)	0.2 ± 0.01
Hausner's ratio	1.3 ± 0.02
% compressibility	25.2 ± 1.86

Viscosity and flow characteristics

The flow behaviour of a 1.0% w/v aqueous dispersion of the polysaccharide gum is shown in Figure 1.

The viscosity of the gum dispersion decreases with an increase in shear rate. This is indicative of pseudoplastic flow behaviour, or shear-thinning. At high shear rates, the decrease in viscosity can be attributed to a decreasing number of chain entanglements (16).

Effect of temperature and electrolytes on viscosity

The effect of temperature on the apparent viscosity of a 0.5% w/v dispersion of the gum at different shear rates is shown in Figure 2. At any given shear rate, the viscosity of the gum decreases with increasing temperature and vice versa. At a given shear stress, polymer chain entanglement/disentanglement determines flow rate (16). Disentanglement causes a decrease in viscosity, and this is promoted by increased temperature.

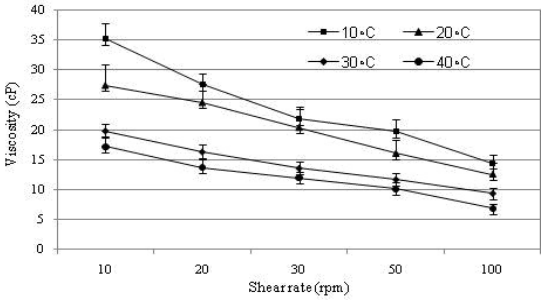

Figure 2 Effect of temperature on the apparent viscosity of grewia gum (n=3, mean ± s.d.).

The effect of electrolyte type and concentration on the apparent viscosity of a 0.5% w/v dispersion of the gum are shown in Figure 3. The viscosity of the gum decreases with increasing concentration of electrolyte.

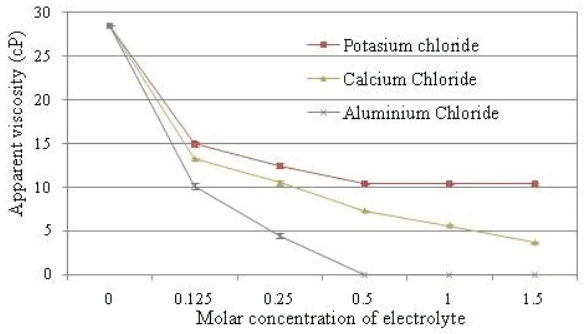

Figure 3 Effect of type and concentration of electrolyte on the apparent viscosity of 0.5% $^{w}/_{v}$ gum dispersion (n=3, mean.

Aluminium chloride is more effective in decreasing the apparent viscosity of the gum dispersion than calcium chloride which was in turn more effective than potassium chloride. The decrease in the viscosity of gums brought about by electrolytes is proportional to the concentration as well as the valence of the cation (17, 28).

Monosaccharide composition by GC analysis

The GC chromatogram of grewia gum is shown in Figure 4. The chromatogram indicates that grewia polysaccharide gum is composed of five neutral sugars eluting at various retention times.

The retention times indicated that grewia gum contains glucose, rhamnose, arabinose, xylose and galactose. The mean relative concentration of these sugars in the polysaccharide is glucose (67.14%), rhamnose (6.2%), xylose (2.72%), galactose (9.61%) and arabinose (12.71%). Okafor (10), using paper chromatography previously reported the gum to be composed of glucose and rhamnose as the only neutral sugars. The use of GC analysis provides a more sensitive detection method.

^{1}H and ^{13}C NMR of grewia polysaccharide gum

The ^{1}H and ^{13}C NMR spectra of grewia polysaccharide gum are shown in Figures 5 and 6 respectively.The ^{1}H NMR spectrum is crowded in a narrow region between 3 to 5 ppm typical of polysaccharides and confirms the presence of many similar sugar residues (19). The 1H NMR spectrum of grewia polysaccharide gum shows the presence of a *C-CH_3* group with signal at 1.15 to 1.21 ppm and further down field, the signal at 1.82 ppm is attributable to *$COOCH_3$* (19). The signals between 3.1 to 4.3 ppm can be assigned to non-anomeric protons (H_2- H_6) while signals between 4.3 to 4.8, and 4.9 to 5.5 ppm arise from β-anomeric and α-anomeric protons respectively (18). The signals between 3.0 to 3.8 ppm have also been assigned to *-O-CH_3*. Again, the *-CH_3* indicates the presence of methylated sugar (rhamnose) and agrees with the ^{13}C NMR.

The ^{13}C NMR spectrum also indicated that the gum contains deoxygenated sugars. This is evident from the *-CH_3* signals appearing in the much higher field 15 to 20 ppm (19). This *-CH_3* is attributable to the methyl group of the rhamnose sugar unit. The signal between 20 to 30 ppm is thought to be a *-CH_2* linked to OH which pushes the signal slightly downfield. The *CH_2OH* may be attributable to the glucose sugar unit. Signals from anomeric carbons of the monosaccharide components appear in the 90 to 110 ppm (19). The α- anomeric carbons are seen in the region of 95 to 103 ppm showing about 5 anomeric carbons which may be attributable to the five neutral sugar components of the polysaccharide while the signals due to non-anomeric carbons C_2-C_5 appear between 60 to 85 ppm.

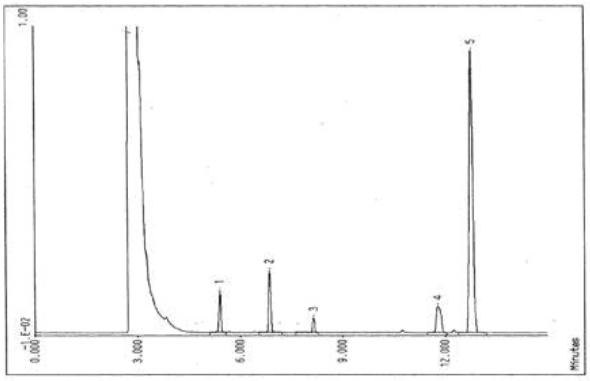

Figure 4 GC chromatograms of alditol acetates from grewia polysaccharide gum after hydrolysis with 2N H_2SO_4.

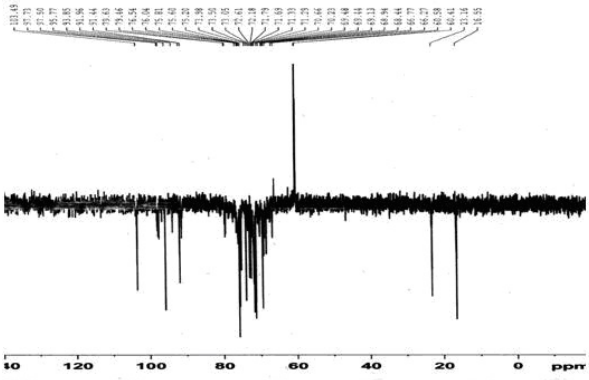

Figure 6 ^{13}C NMR of grewia polysaccharide gum hydrolysed with 2N H_2SO_4 and dissolved in D_2O.

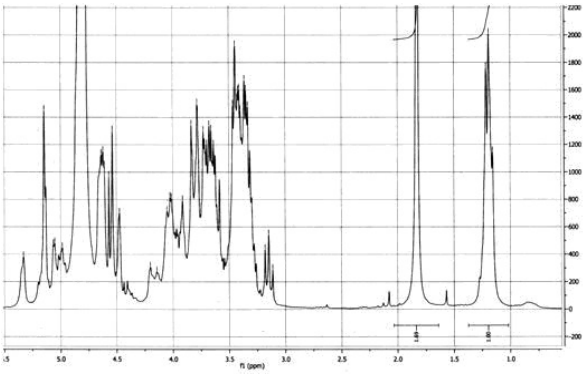

Figure 5 ^{1}H NMR of grewia polysaccharide gum hydrolysed with 2N H_2SO_4 and dissolved in D_2O.

FT-IR spectroscopy

The FT-IR spectroscopic spectrum of grewia gum is presented in Figure 7. The spectra exhibit the typical bands and peak characteristic of polysaccharides. The broad band occurring at 3436 cm^{-1}, results from the presence of hydroxyl (*-OH*) groups. The peak obtained at 2930 cm^{-1} results from stretching modes of the *C-H* bonds of methyl groups (*-CH_3*). Natural gums usually contain fractions of sugar acid units which would usually impart a weakly anionic character to the gum macromolecule (20). Absorption bands around 1618 and 1430 cm^{-1} are typical of carboxylate groups of the galacturonic acid residues (21) as reported by Okafor (10). This region between 1500 and 1800 cm^{-1} is typically used to detect presence of carboxylic groups. Also absorption peaks at 1740 cm^{-1} and 1258 cm^{-1} are typical of acetyl groups (22). The wave numbers between 800 and 1200 cm^{-1} represents the finger print region for carbohydrates (23, 24).

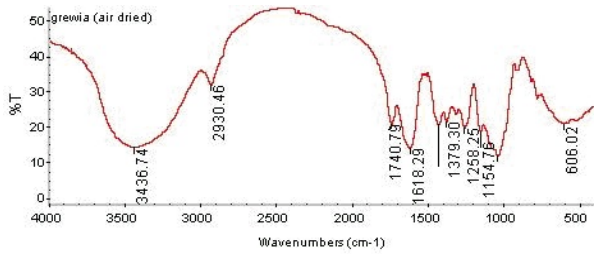

Figure 7 FT-IR spectra of grewia gum showing the various absorption peaks.

Solid-state NMR

Carbon-13 solid-state NMR spectrum of the gum sample is shown in Figure 8.

The spectra give line widths which are typical of an amorphous natural polymer with broad band signal between 64 and 90 ppm arising from the bulk of the ring *C-OH* carbons. The C4 carbon accounts for the high frequency

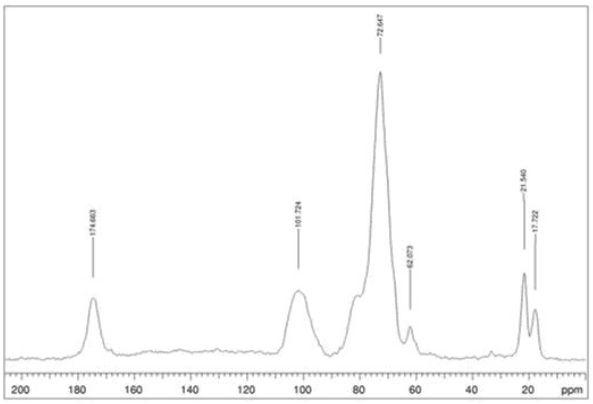

Figure 8 Solid state NMR spectrum of grewia gum.

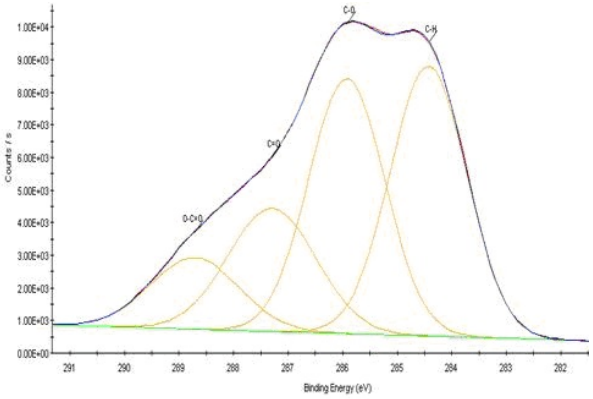

Figure 9 Synthesized carbon 1s scan of grewia polysaccharide gum

shoulder while the C1, anomeric carbons give the signal between 90 and 110 ppm. The shape of this band suggests it is composed of multiple signals but the low resolution suggests the contrary. The low intensity signal at about 62 ppm is attributable to the $–CH_2OH$ belonging to the glucose. The high frequency signal at about 174 ppm is consistent with *–COOH* group and indicates presence of sugar acid. The signals at approximately 18 and 22 ppm may be attributed to the methyl groups from rhamnose. The two signals suggest similar components as seen from the closeness of the signal.

X-ray Photoelectron spectroscopy

XPS gives information on the relative elemental composition (atomic percentage) of all elements on a material's surface except H, and was employed to establish the relative composition of elements in the gum. This technique unlike atomic absorption spectroscopy provides information on the relative amounts of the elements present and not the absolute quantities therein. The elements found in the gum sample were Na, Ca, Mg, N and P. The atomic percentages were Na (trace), Ca (1.7±0.3), Mg (0.8±0.3), N (0.6±0.2) and P (0.1±0.1). The presence of N may indicate the presence of protein impurities in the sample. The synthesized Carbon 1s scan of grewia polysaccharide gum is shown in Figure 9. The relative composition of carbon components of the polysaccharide gum is given by the area under the curve.

Thermal analysis

The representative plot results of thermo gravimetric analysis carried out on the gum under lean oxygen (5% oxygen in nitrogen) atmosphere are shown in Figure 10. The details of thermal behaviour and thermal stability data according to the primary thermograms and derivative thermograms for the gum show that heating at a rate of 10°C per minute from 30°C to a maximum of 600°C results in two mass loss events.

The first mass loss, taking place between 30° - 150°C is attributed to the loss of adsorbed and structural water of biopolymers as related by

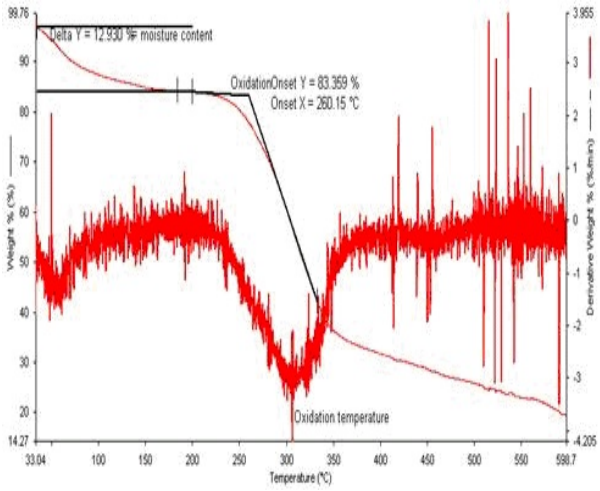

Figure 10 Representative thermo-gravimetric parameter derivatisation for grewia gum.

other authors (9, 25), or due to desorption of moisture as hydrogen-bound water to the polysaccharide structure. The second weight loss event with an onset of about 250°C resulted in a weight loss of about 60%, may be attributed to the polysaccharide decomposition (26, 27) and is described by a weight loss onset of 267±6.2 °C and a maximum oxidation temperature of 318±2.5 °C. The weight loss onset (representing the onset of oxidation or decomposition) of 267±6.2 °C suggests that grewia polysaccharide gum has good thermal stability.

The DSC thermograms of grewia polysaccharide gum, as shown in Figure 11, show two broad endothermic peaks. The peak between 50 and 140°C is attributable to moisture desorption and an a second endothermic transition is seen between 150 and 190 °C.

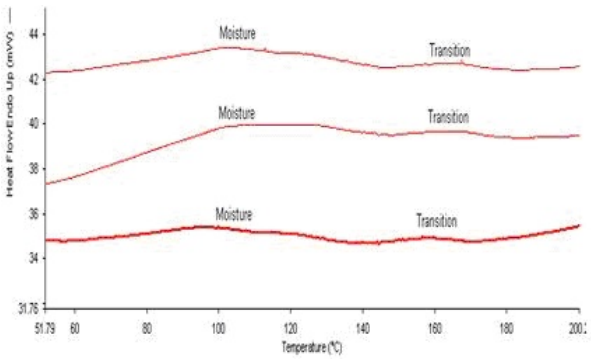

Figure 11 DSC thermograms of grewia polysaccharide gum at 10°Cper minute upto 200°C.

Molecular weight

The molecular weight of grewia polysaccharide gum determined by gel permeation chromatography was expressed as the 'pullulan polysaccharide equivalent' molecular weight. The computed average molecular weights (M_w), number average molecular weight (M_n), and polydispersity (M_w/M_n) were 5925 kDa, 3720 kDa and 1.6 respectively. The accurate determination of the molecular weight characteristics of a polymer is very important and its determination is important in relation to many physical properties of the polysaccharides. The polydispersity index (M_w/M_n) is used as a convenient measure of the range of molecular weight present in a distribution and is in the range of 1.5 – 2.0 for natural polysaccharide gums (16).

Scanning electron microscopy of grewia gum

A scanning electron micrograph of grewia gum is shown in Figure 12. The micrograph is indicative of an amorphous material. The particles are mostly seen as aggregates of irregular shapes and dimensions which are fibrous in nature. The shape and structure or surface topography of the polysaccharide gum may be affected by the method of extraction and purification or preparation of the product (28).

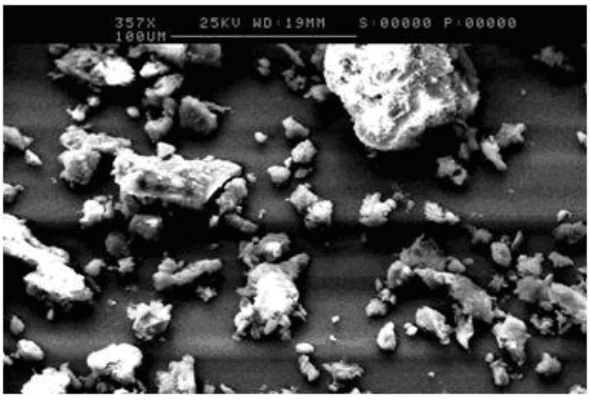

Figure 12 Scanning electron micrograph of grewia gum.

CONCLUSION

Grewia polysaccharide gum has been extracted from the crude pulverised inner stem bark of the plant *Grewia mollis* (Fam. Tiliaceae). The extraction process gave a yield of 32.4% on dry weight basis. Aqueous dispersions of the gum hydrate and swell to form highly viscous dispersions which exhibit pseudoplastic flow (shear-thinning) behaviour. This property can be exploited in the food, cosmetic and pharmaceutical industries. Materials with such properties have therefore been used as

stabilizers and suspending agents in foods, cosmetics and in liquid or solid dosage forms. The high thermal stability of the gum as evidenced by the high oxidation onset of 267±6.2 °C indicates that the gum can be used as excipient even under conditions of high thermal stress.

The natural hydrophilic colloids are widely used in pharmaceutical dosage forms because of their biocompatibility, low cost and relatively free availability (9). The relative abundance and easy availability of grewia polysaccharide gum may reduce cost and save foreign exchange in Nigeria where it is found growing abundantly wild or cultivated and forms part of the food delicacies of the inhabitants of the region.

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REFERENCES

1. Talukdar MM, Vercammen JP. Evalution of xanthan gum as a hydrophilic matrix for controlled-release dosage form preparation. Drug Development and Industrial Pharmacy 19: 1037-1046, 1993.

2. Huber HE, Christenson GI. Utilization of Hydrophilic gums for control of drug release from tablet formulation 1: Disintegration and dissolution behaviour. J. Pharm. Sci., 1: 59-66, 1996.

3. Sujja-Areavath J, Munday DL, Cox PJ, Khan KA. Release characteristics of diclofenac sodium from encapsulated natural gum mini-matrix formulations. International Journal of Pharmaceutics 139: 53-62, 1996.

4. Bhardwaj TR, Kanwar M, Lal R, Gupta A. Natural gums and modified natural gums as sustained-release carriers. Drug Development and Industrial Pharmacy 26(10): 1025-1038, 2000.

5. Varshosaz J, Tavacoli N, Eram SA. Use of natural gums and cellulose derivatives in production of sustained release metoprolol tablets. Drug Delivery 13(2): 113–119, 2006.

6. Udeala OK, Chukwu A. The binding property of mucuna gum in sulphadimidine and chloroquine phosphate tablets. Nigeria Journal of Pharmaceutical Sciences 1: 59-66, 1985.

7. Bilany, M.R., Suspensions and emulsions, in Aulton ME (ed), Pharmaceutics: the design and manufacture of medicines. 3rd ed., *Churchill Livingstone,* Philadelphia, pp 383-405, 2007.

8. Middleton, D.L., Leung, S.H.S., Robinson, J.R., Ocular bioadhesive delivery systems, in Leenaerts V; Gurny R (eds), Bioadhesive drug delivery systems. *CRC Press INC,* Boca Raton, Florida, pp 179-202, 1990.

9. Vendruscolo CW, Ferrero C, Pineda EAG, Silveira JLM, Freitas RA, Jimenez-Castellanos MR, Bresolin TMB. Physicochemical and mechanical characterization of galactomannan from Mimosa scabrella: Effect of drying method. Carbohydrate Polymers 76: 86-93, 2009.

10. Okafor IS, Chukwu A, Udeala K. Some physicochemical properties of grewia gum. Nigeria Journal of Polymer Science and Technology 2(1): 161-167, 2001.

11. Emeje M, Isimi C, Kunle O. Effect of grewia gum on the mechanical properties of paracetamol tablet formulations . African Journal of Pharmacy and Pharmacology 2(1): 001–006, 2008.

12. Nep EI, Okafor IS. Evaluation of the bioadhesive property of grewia gum in indomethacin tablet formulation in pig gastric mucus. Journal of Pharmacy & Bioresources 3 (2): 62-69, 2006.

13. Muazu J, Musa H, Musa KY. Compression, mechanical and release properties of paracetamol tablet containing acid treated grewia gum. Journal of Pharmaceutical Science and Technology 1 (2): 74-79, 2009.

14. AOAC (Association of Official Analytical Chemists). Official Methods of Analysis (15th Ed). Washington DC: Association of Official Analytical Chemists, 1990.

15. Tomoda M, Satoh N, Suzuki Y. Plant mucilages XV. The main structural features of the backbone chain of paniculatan. Chem. Pharm. Bull, 25: 968-973, 1977.

16. Qi, W., Cui SW., Understanding the physical properties of food polysaccharides, in Cui SW (ed), Food Carbohydrates: Chemistry, Physical Properties, and Applications. *Taylor and Francis,* Boca Raton, Florida, pp 161-262, 2005.

17. Vernor-Carter EJ, Sherman P. Rheological properties and application of mesquite tree (Propolis juliflora) gum 1: Rheological properties of mesquite gum Solution. Journal of Texture Studies, 11: 339-349, 1980.

18. Glicksman, M., Schachat, R.E. Industrial gums, in whistler RL (ed.), The chemistry of plant gums and mucilages. *Academic press,* New York, NY, pp 213-298, 1959.

19. Cui, S.W., Structural analysis of polysaccharides, in Cui SW (ed.), Food Carbohydrates: Chemistry, Physical Properties, and Applications. *Taylor and Francis,* Boca Raton, Florida, pp 105-157, 2005.

20. Wang Q, Ellis PR, Ross-Murphy SB. Dissolution kinetics of guar gum powders 2: Effects of concentration and molecular weight. Carbohydrate Polymers 53: 75-83, 2003.

21. Figueiro SD, Goes JC, Moreira RA, Sombra ASB. On the physicochemical and dielectric properties of glutaraldehyde cross-linked galactomannan-collagen films. Carbohydrate polymers 56: 313-320, 2004.

22. Xiaodong M, and Marek P. Intrinsic Viscosities and Huggins Constants of Guar gum in Alkali Metal Chloride Solutions. Carbohydrate Polymers 70 (1): 15-24, 2007.

23. Fillippove MP. Practical infrared spectroscopy of pectin substances. Food Hydrocolloids 6: 115-142, 1992.

24. Cui SW, Phillips GO, Blackwell B, Nikiforuk J. Characterization and the properties of Acacia senegal (L.) Willd. Var.senegal with enhanced properties (Acacia (sen) SUPERGUM^{TM}): Part 4. Spectroscopic characterization of Acacia Senegal var. Senegal and Acacia (sen) SUPERGUM^{TM} Arabic. Food Hydrocolloids 21: 347-352, 2007.

25. Kittur FS, Harish Prashanth KV, Udaya Sankar K, Tharanathan RN. Characterization of chitin, chitosan and their carboxymethyl derivatives by differential scanning calorimetry. Carbohydrate Polymers 49: 185–193, 2002.

26. Varma AJ, Kokane SP, Pathak G, Pradhan SD. Thermal behaviour of galactomannan guar gum and its periodate oxidation products. Carbohydrate Polymer 32: 111-114, 1997.

27. Zohuriaan MJ, Shokrolahi F. Thermal studies on natural and modified gums. Polymer Testing 23: 575-579, 2004.

28. Qian J, Chen W, Zhang W, Zhang H. Adulteration identification of some fungal polysaccharides with SEM, XRD, IR and optical rotation: A primary approach. Carbohydrate Polymers 78: 620–625, 2009.