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Isolation and Characterization of Bionanofibers from *Moringa Oleifera* Gum as a Platform for Drug Delivery

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Abstract Bionanofibers were isolated from *Moringa Oleifera* gum by carrying out combination of acid hydrolysis and probe sonication treatment. Acid hydrolysis of hemicelluloses was carried out to remove amorphous region. An increase in sulphuric acid concentration from 20 to 60 wt% accelerates the breakages in hemicelluloses molecules, leading to narrower, less polydisperse bionanofibers. After sonication, the fibers were cleaved into small fibers with dimensions on the nanoscale. The characterization of bionanofibers was performed by Scanning Electron Microscopy (SEM), Particle size, Differential Scanning Calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR). Surface morphology was observed by SEM which confirms presence of bionanofibers. Bionanofibers having diameter at 98 nm while length of 20 µm. The thermal analysis of *Moringa oleifera* gum was done by DSC. Fourier transform infrared spectroscopy (FTIR) showed that some breakages of intramolecular hydrogen bonds and glycosidic bonds held during hydrolysis reaction of Moringa Oleifera. The isolated nanofibers can be used in novel drug delivery system as platform to load the desired drug.

Keywords: *Moringa Oleifera gum, Hemi cellulose, Acid hydrolysis, Ultra sonication, Bionanofibers etc*

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1. Introduction

Polymers are playing an important role in the world for a number of applications beginning from daily needs to defense and biomedical areas. Polymers obtained from different biorenewable resources are generally called as bio based polymers and they possess a well-defined structure such as natural fibers, starch, proteins etc. Polysaccharides are the most plentiful natural polymers mainly extracted from agricultural and biomass resources [1].

Nanofibers obtained from bio based polymers having potential applications in drug delivery, medicine, environmental protection, agriculture, production and processing of food [2]. It's having unique characteristics such as high tensile strength, high surface area-to-volume ratio, high Young's modulus and low coefficient of thermal expansion [3]. Polysaccharide nanofibers have widely used because of its availability, biocompatibility, their mechanical strength, naturally optimized structure, biodegradability, sustainability and environmental friendliness [4]. Cellulose is one of the most important biopolymers and its give nanofibers with a diameter that range from 2-20 nm and a length in few micrometers [5].

Compared with cellulose, hemicelluloses are the second most sources of naturally occurring polymers. Hemicelluloses consist of a group of heteropolysaccharides as pentose's (xylose, arabinose) and hexoses (mannose, glucose, and galactose) according to their structure [6]. Polysaccharides obtained from plant gums which are oozes from many trees and plants when their barks are injured and having high molecular weight, either soluble in water or which can be at least dispersed in it [7].

Moringa gum is a biobase natural polymer obtained from bark of *Moringa oleifera* trees belonging to family Moringaceae. Gum is easily collected and also available in huge amount. The purified gum exudate obtained from drumstick plant contains polysaccharide units as D- xylose, L- arabinose, D- galactose, L- rhamnose, D- mannose as pentose's and hexoses respectively [8].

Several studied have reported that Moringa gum find it's applications in different fields such as sustained release polymer [9], tablet disintegrant [10], gelling agent, suspending agent, film forming property [11], binder and release retardant [12], and having emulsifying properties [13].

2. Materials and method

2.1. Materials

Moringa oleifera gum (collected from trees), Conc. Hydrochloric acid (wt. %), Bial's Orcinol reagent, Tollen's reagent, aniline acetate.

2.2. Isolation of *Moringa Oleifera* gum

The gum was collected from injured site of trees. Gum was dried, ground and passed through sieve no. 80. Dried gum was added in distilled water and stirred for 6-8 hours at room temperature. By centrifugation supernatant was obtained. The residue was washed with water and the washings were added to supernatant, the washing was repeated at least four times. By continuous stirring the supernatant was made up to 500 ml and treated with twice the volume of acetone. The precipitated material was washed with distilled water and dried at 50-60°C [14].

Confirmation of hemicelluloses (pentose's and hexoses) in *Moringa Oleifera* gum were identified by Bial's Orcinol test, Aniline acetate test for pentose's sugar and Tollen's pholoroglucinol test, Cobalt chloride test for hexose sugar's. The confirmatory test for hemicelluloses was positive [15].

2.3. Extraction of Bionanofibers

Table 1. Experimental Design of Bionanofibers

Sr. No.	Batch	H2SO4Conc. (Wt. %)	RPM	Hydrolysis Time (hrs.)	Temperature(°C)
1	F_1	20	5,000	1	40
2	F_2	20	7,500	2	40
3	F_3	20	10,000	3	40
4	F_4	40	5,000	2	40
5	F_5	40	7,500	3	40
6	F_6	40	10.000	1	40
7	F_7	60	5,000	3	40
8	F_8	60	7,500	1	40
9	F_9	60	10,000	2	40

Bionanofibers were produced by combination of acid hydrolysis and ultrasonic treatment from *Moringa oleifera* gum obtained above. One gram of powder was placed in 100 ml of aqueous solution of sulfuric acid with 20, 40, 60 wt. %. The mixtures was placed on a mechanical stirrer with hot plate, which was heated to 40°C for hydrolysis at 1, 2, 3 hrs. After reaching the reaction time, about 20°C of distilled water was added to stop the reaction. By centrifugation (REMI C-30BL) sulfuric acid was partially removed from resulting suspension with a speed of 5000, 7500, 10,000 rpm for 10 min. Then, the sample was suspended against distilled water until the pH reached 7. Next the resultant suspension was treated with a probe sonicator (Model No. 750FL) at 20-25KHz for 15 min at an out power of 650 W to isolate nanofibers in an ice bath [16,17].

3. Characterization of Bionanofibers

3.1. Surface Morphology

The morphology of the nanofibers was analyzed using scanning electron microscopy (Philips XL30 ESEM). The samples were coated with carbon unit before examination.

3.2. Particle size analysis

The particle size of bionanofibers was determined by Malvern Zetasizer (Nano ZS 90, UK) using water as dispersion medium. This technique yields the mean particle diameter and the range of particle size distribution (Polydispersity index, PDI).

3.3. Differential Scanning Calorimetry

Differential Scanning Calorimetry (STAR SW 10.00 instrument) was performed under nitrogen atmosphere used to measure physical state i. e. crystalline or amorphous nature of sample.

3.4. Fourier Transformed Infrared Spectroscopy

The FTIR spectra were recorded on a Fourier transform infrared spectrophotometer (8400S, SHIMADZU) in the range of 600-4000 cm^{-1}. A small quantity of each specimen was blended with KBr and compressed to form a disk.

4. Results and Discussion

4.1. Morphological Analysis

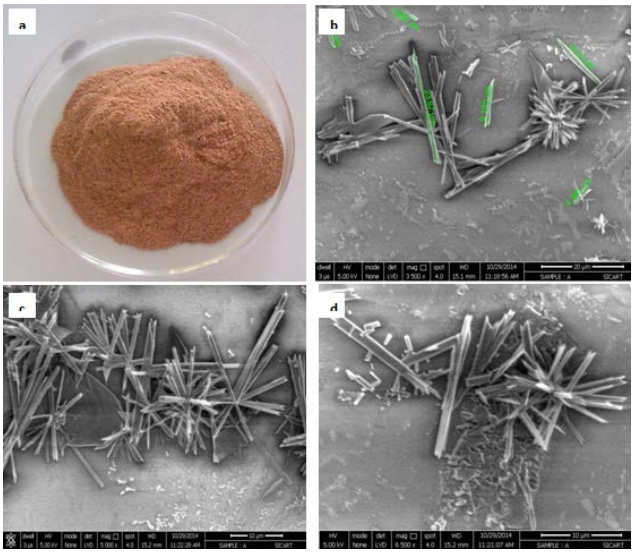

Figure 1. (a) *Moringa Oleifera* gum powder, (b) (c) (d) SEM images of bionanofibers

Surface morphology is shown in figure (b) (c) (d). SEM micrograph shows bundles of fibers having diameter at 98 nm and length of 20 µm. *Moringa oleifera* gum is naturally occurring polymer that is obtained through hydrolysis using dilute mineral acids. After chemical pretreatment, the fibers were separated into individual micro-sized fibers; these micro-sized fibers were composed of strong hydrogen bonding.

4.2. Particle Size

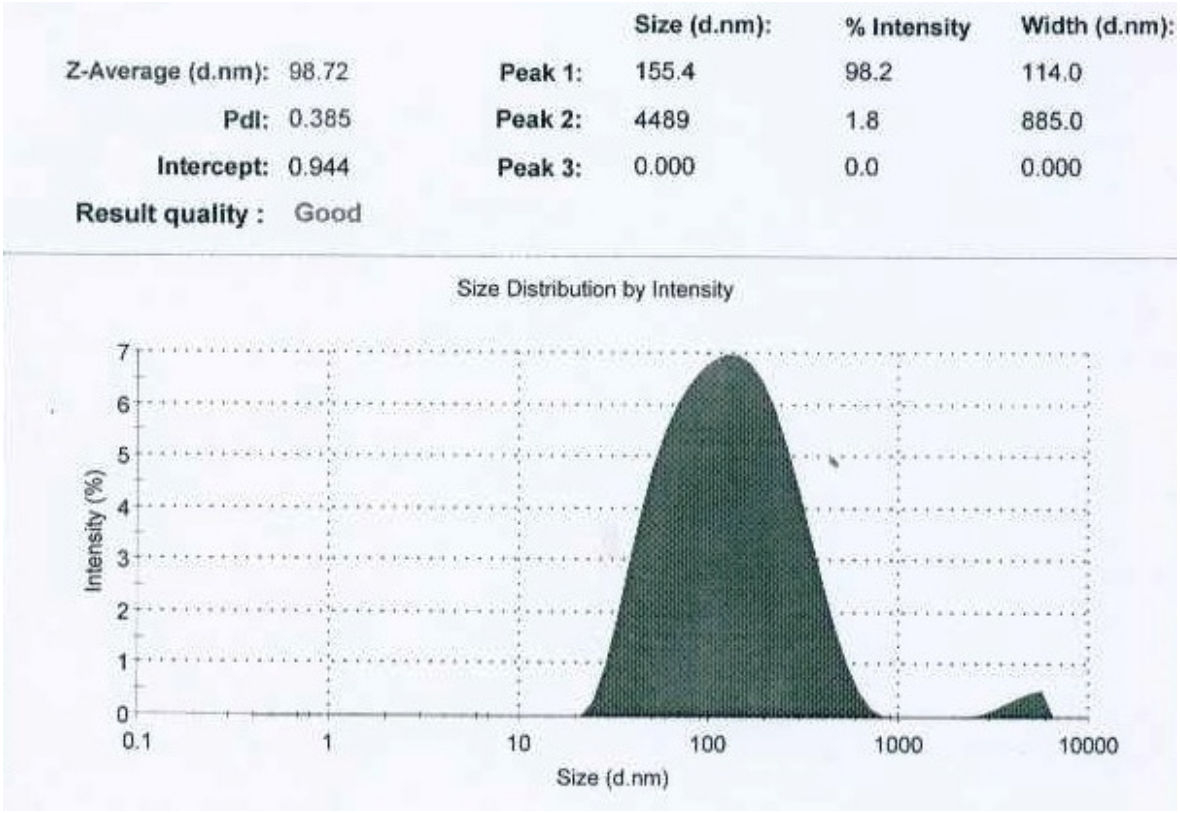

Figure 2. Particle size distribution of bionanofibers

The physical stability of bionanofibers depends upon the particle size and particle size distribution, dynamic light scattering measurement showed that peak particle size of bionanofibers were approximately within range. The average particle size was found to be 98 nm with diameter. The particle size distribution graph was found to be bell shaped with even distribution range. Higher the polydispersibility index then lowers the uniformity of the particle size. Polydispersibility index found to be 0.5 which indicates the particles were in monodisperse form.

4.3. Differential Scanning Calorimetry

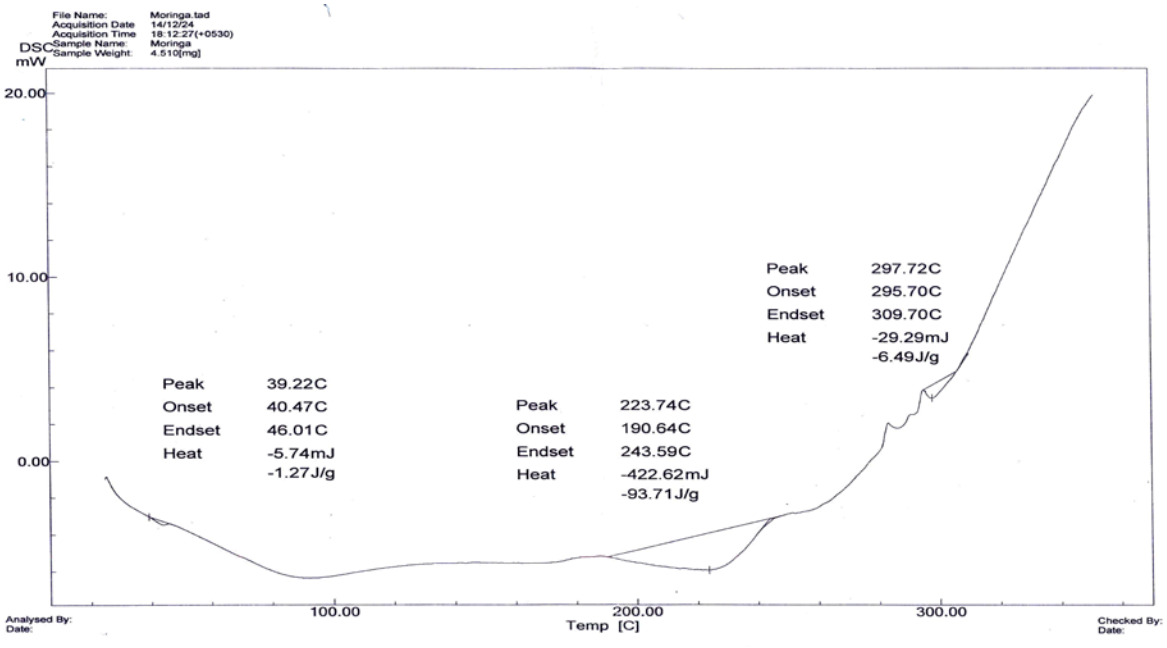

Figure 3. DSC pattern *of Moringa Oleifera* gum

Differential Scanning Calorimetry (DSC) measure heat loss or gain resulting from physical or chemical changes with sample as a function of temperature. The endothermic peak usually indicates the loss of water present in compound. The DSC thermogram of *Moringa Oleifera* gum is shown in Figure 3 MOG shows glass transition appears at 39.22°C depends on the materials natural characteristics such as structure, bonding and molecular weight. The endothermic peak of polymer showing at 223°C. This is enthalpy of transitions and associated with the crystallinity of materials.

4.4. Fourier Transformed Infrared Spectroscopy

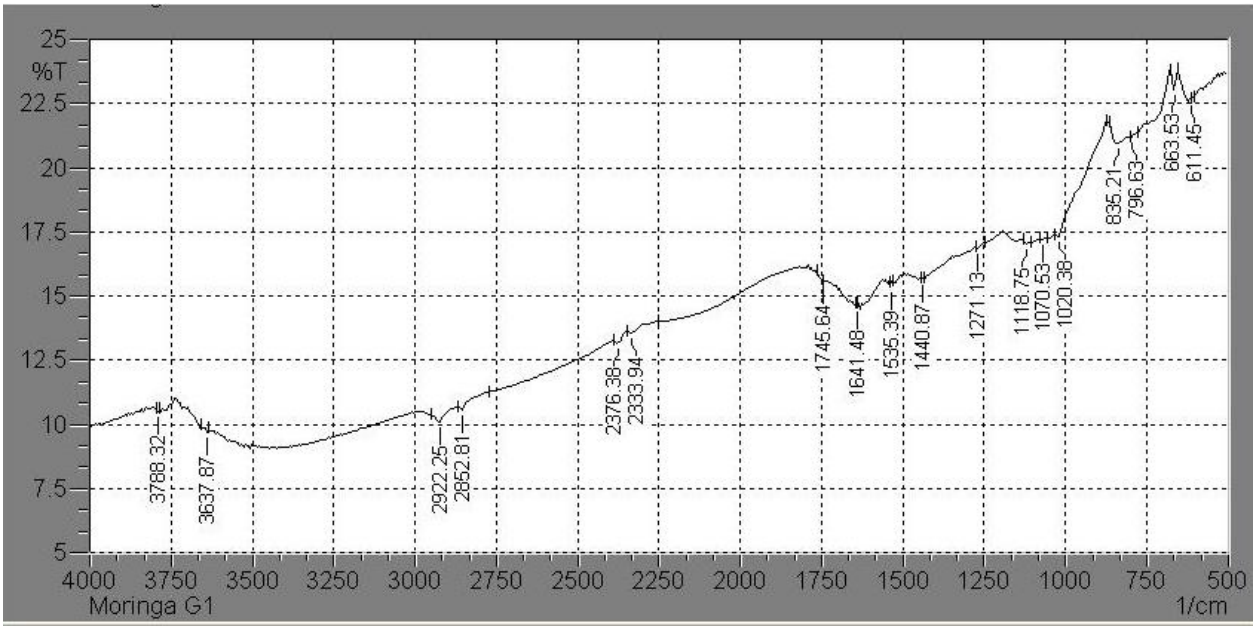

Figure 4. FT-IR of Moringa Oleifera gum

The Fourier Transformed Infrared Spectroscopy (FT-IR) spectrum of *Moringa Oleifera* gum was presented in Figure 3. In spectrum the attribution of main absorptions were the characteristic of polysaccharide structures and related to OH stretching (3637 cm^{-1}); CH stretching (2922 cm^{-1}); C=C alkenes aromatic (1641 cm^{-1}); C-N amine (1070 cm^{-1}); CHO stretching (1745 cm^{-1}); C-O-C stretching (1020 cm^{-1}) [18,19].

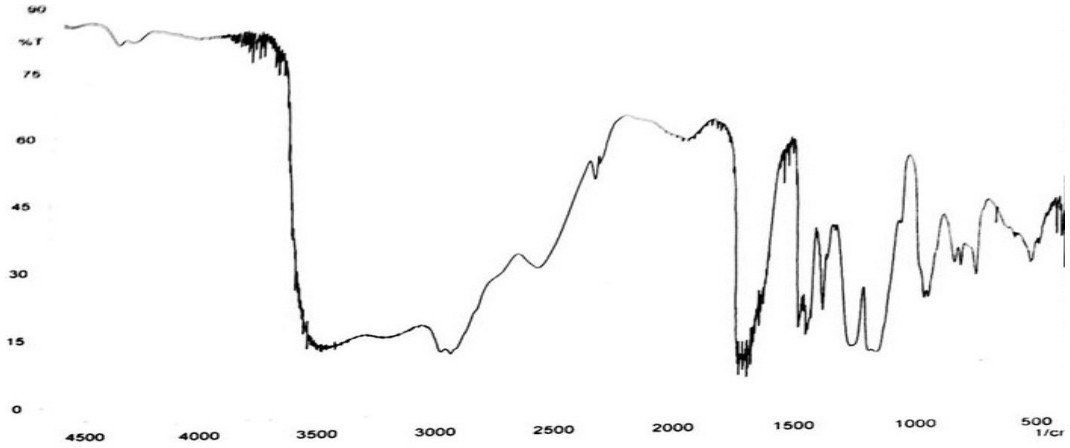

Figure 5. FT-IR of isolated bionanofibers

Figure 5 shows the absorption peaks at 3669 to 3674 cm^{-1} as OH stretching, 2932 cm^{-1} as CH stretching, 1645 cm^{-1} as C=C alkenes aromatic, 1065 cm^{-1} as C-N amines, 1738 cm^{-1} as CHO stretching confirming the polysaccharide structures [19].

5. Conclusion

Bionanofibers were successfully isolated from *Moringa Oleifera* gum using acid hydrolysis and ultrasonication treatment, and further characterized by surface morphology and particle size of obtained bionanofibers. It has been observed that an increase in acid concentration from 20 to 60 wt% accelerates breakage in molecules, leading to narrower, less polydisperse nanofibers. Bionanofibers showing diameter at 98 nm while length of 20 µm. The DSC shows endothermic peak at 223°C of polymer. The FTIR shows some breakage of hydrogen and glycosidic bonds present in polysaccharides during hydrolysis reaction. This developed method is simple, reproducible and does not require sophisticated equipment like elctrospinning machine or nanospider. The isolated nanofibers can be used in novel drug delivery system as platform to load the desired drug.

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