

## Optimization and Characterization of Purified Polysaccharide from *Terminalia Belarica* Gum as Pharmaceutical Excipient

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### Abstract

Natural polysaccharides are drawing a great deal of attention in the recent years as excipient in the pharmaceutical industry. Due to the advances in drug delivery technology, natural polysaccharides are included in controlled drug delivery to fulfill multitask functions and in some cases directly or indirectly influence the extent and /or rate of drug release. In the present study, the polysaccharides extracted from *Terminalia belarica* gum (TBG) were evaluated for its safety and potency towards pre formulation studies as a pharmaceutical excipient. The study was undertaken with an objective to expound the physicochemical, thermal and functional properties of polysaccharide obtained from *Terminalia belarica* gum (TBG). A purified component of polysaccharide was isolated from *Terminalia belarica* gum (TBG) by gel filtration method. Elemental analysis, Scanning electron microscopy (SEM), X-ray diffraction spectroscopy (XRD), Differential scanning microscopy (DSC) and Fourier-transformed infrared (FT-IR) techniques were used to characterize the polysaccharide. Elemental analysis (CHN analysis) of the polysaccharide has shown the contents of carbon, hydrogen and nitrogen contents to be 39.31, 5.56 and 0.18 (w/w %) respectively. SEM analysis suggests that the polysaccharide has irregular particle size, mostly seen in aggregates and fibrous in nature. The XRD pattern of the polysaccharide indicates both crystalline and amorphous structure. The experimental work provides enough evidence to exploit this natural biopolymer in food and pharmaceutical industry.

**Keywords:** *Terminalia belarica* gum (TBG), Gel filtration method, Polysaccharide, Elemental analysis.

### Introduction

In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of plants used in various pharmaceutical application.<sup>1</sup> Plant derived polysaccharides have evoked tremendous interest due to their diverse pharmaceutical applications such as diluent, binder, disintegrant in tablet formulation, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository.<sup>2</sup> These polymers of monosaccharide are inexpensive and available in a variety of structures with a variety of properties. They are highly stable, safe, non-toxic and hydrophilic and gel forming in nature. Since these biopolymers are copious, come from renewable sources, are relatively inexpensive, are non toxic and are amenable to both chemical and biochemical modification, it is not astonishing that they find pervasive and extensive use.<sup>3</sup> They have also been found useful in formulating immediate and sustained release preparations.<sup>4</sup>

*Terminalia belarica* plant is growing widely throughout the Indian subcontinent, Sri Lanka and South-East Asia. In the Traditional system of medicine like Ayurveda, Siddha and Unani, its medicinal uses have been described in several diseases and the action in almost all system. Glucoside, Tannins, Gallic acid, Ellagic acid, Ethylgalate, Gallylglucose, Chebulanic acid is mainly believed to be responsible for its various therapeutic actions. It is used as antioxidant, antimicrobial, anti diarrheal, anticancer, anti diabetic, antihypertensive, hepatic protective and immune stimulatory agent.<sup>5</sup> The gums of the plant have been used for the formulation of microencapsulated drug delivery system.<sup>6</sup>

The term 'gum' most often specifically denotes a group of industrially useful polysaccharides (glycans) or their derivatives that hydrate in hot or cold water to form viscous solutions or dispersions at low concentrations. The extraction and characterization of non starch polysaccharide from Gum is an essential step in establishing their suitability as pharmaceutical excipient.

Owing to their salable usefulness, physicochemical characterization of these polysaccharides is of substantially importance. Literature survey reveals that comprehensive physicochemical characterization and evaluation of the polysaccharides isolated from *Terminalia belarica* gum (TBG) as pharmaceutical excipient has not been performed. Therefore the objective of this study was to isolate and purify the polysaccharide from *Terminalia belarica* gum (TBG) and examine the various pharmaceutical properties to assess its functionality as an excipient. Specifically, the study evaluated the physicochemical properties including flow characteristics, elemental composition and thermal behaviors. The isolated polysaccharide may provide an alternative to other natural polysaccharide or their synthetic counterparts to design and formulate suitable novel drug delivery system.

## Materials and methods

### Materials

*Terminalia belarica* gum (TBG) was obtained from Similipal forest of Odisha. Levamisole was kindly gifted by Wockhardt Limited, Baddi, India. DEAE- Sephadex A-50 was purchased from Hi-media, Mumbai, India. Glucose, d-glucuronic acid, was purchased from S.D fine chemicals, Mumbai, India. All other chemicals and solvents used were of analytical grade.

### Extraction, isolation and purification of polysaccharides

Dried gums were collected from the trunk bark of *Terminalia belarica* plant and separated from fungal affected and other foreign object present during collection. They were converted into small pieces using a hammer. The pieces were then dried in oven dryer maintained between 40°C to 50°C for 72 h to obtain dry mass which were milled with a grinder to get the dry powder. The dried powder was passed through sieve number 40# and stored in an airtight container. The powder (1 kg) was extracted with boiling water three times. The aqueous extract was filtered through Whatman filter paper. The filtrate was concentrated in a rotary evaporator under reduced pressure, and then centrifuged at 3000 rpm for 15 min. The supernatant was precipitated with three volumes of acetone, and stored overnight at 4°C. The precipitate was collected and washed again three times with acetone to get crude polysaccharide (60.4 g). The crude polysaccharide was subjected to DEAE- Sephadex A-50 column chromatography, washed with H<sub>2</sub>O and eluted with 1.0 M NaCl solution. Most of the pigments were absorbed in the column. The Elutes collected were concentrated under reduced pressure to an appropriate volume, and then dialyzed against distilled water. The

remaining portion was lyophilized to afford total purified polysaccharide (yield: 28.4 g).<sup>7</sup>

### Composition of polysaccharides

Total sugar content was estimated by the phenol-sulfuric acid analysis using glucose as standard.<sup>8</sup> The nature of the carbohydrate was confirmed by Molisch tests, Fehling's test and Iodine test. The total carbohydrate content was determined by anthrone method. The protein content was estimated by UV-VIS spectra and as per the method described by Bradford test using bovine serum albumin as standard.<sup>9</sup> Uronic acid content was determined by the carbazole-sulfuric acid method using d-glucuronic acid as standard.<sup>10</sup>

### Solubility test

The purified polysaccharide obtained from *Terminalia belarica* gum (TBG) was evaluated for solubility in water, acetone, chloroform, and ethanol in accordance with the B.P specification.<sup>11</sup>

### Loss on Drying

500 mg of polysaccharide (TBG) was weighed and placed in a clean and neat china dish. It was kept in hot air oven at 105°C until a constant weight was obtained. The china dish was removed from the oven and again the weight of the polysaccharide powder was determined.<sup>12</sup> The moisture content was then determined as the ratio of weight of moisture loss to weight of sample expressed as a percentage.

### Total ash and acid insoluble ash

2 g of polysaccharide (TBG) was weighed accurately in a previously ignited and tarred silica crucible. The material was then ignited by gradually increasing the heat to 500-600°C until it appeared white indicating absence of carbon. It is then cooled in a dessicator and total ash in mg per gm of air dried material is calculated. To the crucible containing total ash, 25ml of 2M HCl was added and boiled gently for 5 minutes, and then about 5ml of hot water was added and transferred into crucible. The insoluble matter was collected on an ash less filter paper. This was then washed with hot water until filtrate is neutral and the filter paper along with the insoluble matter was transferred into crucible and ignited to constant weight. The residue was then allowed to cool and then weighed.<sup>13</sup> The percentage of acid insoluble ash was calculated from the weight of the sample taken.

### Determination of pH

This was done by shaking a 1 % w/v dispersion of the sample in water for 5 min and the pH determined using a

digital pH meter. The data present here is for triplicate determination.<sup>14</sup>

### Angle of Repose

The polysaccharide (TBG) 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 following equation:

$$\text{Angle of repose } \theta = \tan H/R$$

Where  $\theta$  = Angle of Repose  
 $h$  = Weight of powder heap  
 $r$  = Radius of powder heap

### Bulk and tapped densities

Polysaccharide (TBG) was accurately weighed (10 gm) 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 (Electro lab, model ETD-1020). The volume of the powder was read after every 25 taps up to a total of 275 taps when volume of powder was constant. This represents the tapped volume of the powder. The bulk density and tapped density was calculated using the following equations.

$$\text{Bulk Density } (\rho_b) = \frac{\text{Weight of sample}}{\text{Bulk volume}}$$

$$\text{Tapped Density} = \frac{\text{Weight of sample}}{\text{Tapped Volume}}$$

### Compressibility index

The compressibility index was calculated as follows

$$\text{Compressibility } (C \%) = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}}$$

### Hausner ratio

Hausner ratio is a measure of flowability of the TBG polysaccharide and is calculated using the following equation. A low Hausner ratio means that the polysaccharide powder has a high flowability. Hausner ratio above 1.25 indicates poor flow.

$$\text{Hausner ratio } (H) = \frac{\text{Tapped density}}{\text{Bulk density}}$$

### Kawakita analysis

The Kawakita equation is described by using the following equation. This equation describes the relationship between the degree of volume reduction of the powder column and the applied pressure.<sup>15</sup> The basis for the Kawakita equation for powder compression is that particles subjected to a compressive load in a confined space are viewed as a system in equilibrium at all stages of compression, so that the product of the pressure term and the volume term is a constant

$$\frac{P}{C} + \frac{P}{a} = \frac{1}{ab}$$

Where *C* is the degree of volume reduction of a powder compact at pressure *P* (no of tapping) “*C*” can be expressed as  $C = V_0 - V_\infty / V_0$ , where  $V_0$  is the initial volume before tapping and  $V_\infty$  is the final tapped volume after ‘*n*’ number of tapping. The constants (*a* and *b*) can be evaluated from a plot of *P/C* versus *P*. A value of “*a*” is indicative of the total volume reduction for the powder bed, and *b* is a constant that is inversely related to the yield strength of the particles. The data from this study were modeled via the Kawakita equation in an attempt to evaluate the relationship between the volume reduction and applied pressure for under studied isolated polysaccharide as pharmaceutical binder.

### Swelling Index

This was done by taking 1.0 g quantity of in a 15 ml plastic centrifuge tubes and the volume occupied was noted. Ten milliliters of distilled water was added to it and the content was mixed on a vortex mixer (Labline Equipments, India) for 2 min. The mixture was allowed to stand for 10 min and immediately centrifuged at 1000 rpm on a bench centrifuge (Remi, India). The supernatant was carefully decanted and the volume of sediment was measured.<sup>16</sup> The swelling index was computed using the following equation.

$$S = (V_2 - V_1) / V_1 \times 100$$

Where *S* is the % swelling capacity,  $V_2$  is the volume of the hydrated or swollen material and  $V_1$  is the volume of the material prior to hydration. The experiment was repeated by using 0.1 N HCl and Phosphate buffer 7.4 in water.

### Viscosity and Effect of ageing on viscosity of polysaccharide

The viscosity of a 1.0% w/v dispersion of the polysaccharide obtained from *Terminalia belarica* gum (TBG) read at shear rates between 0.1 to 10.0 reciprocal

seconds and at 23°C using a Brookfield viscometer (LVDV-E) (Brookfield Engineering Labs, Stoughton-USA). Spindle 62 was used and 3 minutes was allowed for stabilization of the readings before the viscosity was read.

To study the effect of ageing on viscosity of , the above was stored in a controlled humidity and temperature (75 % RH and 40°C ) environment for 180 days after which the viscosity experiment was repeated and the average results recorded.<sup>11</sup>

### Microbial quality

The total aerobic viable count and fungal count of polysaccharide obtained from *Terminalia belarica* gum (TBG) was determined by the pour - plate method.<sup>17, 18</sup> In determining the presence of pathogenic bacteria and fungus in the polysaccharide, 0.1 g of the powder was dissolved separately in 10 ml of sterile water and 1ml of the solution was inoculated into a previously stabilized casein soya bean digest agar and Sabouraud plus streptomycin agar respectively. Four specific pathogens viz. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella spp.* were checked for their presence alongwith total aerobic bacterial count and combined yeast and mould count. The inoculated agars were incubated at 37°C for 48 hours and growth of specific organisms depending on the selective media that was used was read as present or not present. All the experiments were done in triplicate.

### Elemental Analysis

Elemental analysis of carbon, hydrogen and nitrogen was carried using a Perkin Elmer 2400 Semis II CHN analyzer. Accurately weighed 0.5 mg of sample was heated to 1150 °C and the corresponding element was determined by using thermal conductivity detector.<sup>19</sup>

### Scanning Electron Microscopy

The morphological features of the T.B.G were studied with HITACHI, S-3600 N Scanning Electron Microscope. The dried sample of polysaccharide obtained from *Terminalia belarica* gum (TBG) was mounted on a metal stub and sputtered with gold in order to make the sample conductive, and the images were taken voltage of 15 KV at 100 x and 500x magnifications respectively.

### Differential scanning calorimetry

A differential scanning calorimeter (JADE DSC, Perkin Elmer, and USA) was used to study the thermal properties of the polysaccharide obtained from *Terminalia belarica* gum (TBG). The polysaccharide was scanned in the temperature range of 50-220°C under an atmosphere of

nitrogen. The heating rate was 20°C/min, followed by a cooling cycle back to 30°C at the same rate.

### X-ray powder diffraction

X-ray diffraction (XRD) patterns of the polysaccharide obtained from *Terminalia belarica* gum (TBG) were analyzed using a Siemens D5000 Xray diffractometer (Siemens, Munich, Germany). Powder sample, packed in rectangular aluminium cells, illuminated using CuK $\alpha$  radiation ( $\lambda = 1.54056 \text{ \AA}$ ) at 45 kV and 40 mA. Samples were scanned between diffraction angles of 5° to 40°C 2 $\theta$ . Scan steps of 0.1 were used and the dwell time was 15.0 Sec. A nickel filter was used to reduce the K $\beta$  contribution to the X-ray signal. The 'd' spacing was computed according to Bragg's law of diffraction.

### Fourier transform-infra red

The Fourier transform-infra red (FT-IR) spectrum of the sample was recorded in an IR spectrometer (Bruker Alpha FTIR). Triplicate measurements were made, and the spectrum with the clearest identifiable peaks was chosen.

### Statistical analysis

Data were expressed as mean  $\pm$  standard deviations of three replicated determinations.

### Result and Discussion

Polysaccharide is mixture of a number of different macromolecular substances and the yield and composition of polymer can vary depending on the methods of isolation.<sup>20</sup> The yield of total isolated purified polysaccharide (TBG) was 2.84% of the raw material. TBG polysaccharide showed negative Fehling's reagent and iodine-potassium iodide reactions, indicating that it did not contain reducing sugar and starch type polysaccharide. The total polysaccharide content was estimated to be 89%. The UV-Vis spectra showed that the polysaccharide had an absorption peak at 190 nm only, which is the characteristic UV absorption peak for a polysaccharide. There was no absorption at 260 and 280 nm, indicating that the polysaccharides contained no protein or polypeptide. Bradford test was confirmed the absence of protein in TBG polysaccharide. The uronic acid content in TBG was found to be 4.3%. Physicochemical properties of the TBG are summarized in Table 4.

The polysaccharide is soluble in water and practically insoluble in ethanol, acetone and chloroform. The results of the swelling characteristics show that TBG has high swelling index suggesting that the polysaccharide may perform well as binder/disintegrant/ matrixing agent. The swelling was highest in water followed by phosphate



buffer and least in 0.1 N HCl. The results indicate that the polysaccharide is a pH responsive polymer and may find application in controlled release formulations.<sup>21</sup> The moisture content of TBG polysaccharide was low. This knowledge is essential for designing and optimizing many process involved in production using these polysaccharide like drying, packing and storing. Given suitable temperature moisture will lead to the activation of enzymes and the proliferation of micro organisms, thereby affecting the shelf life of most routine formulations. Therefore, the low moisture content value of TBG polysaccharide indicates its suitability in formulations containing moisture sensitive.<sup>22</sup>

The total ash and acid insoluble ash value of TBG polysaccharide was found to be 5.8 and 0.08%w/w respectively. Ash values reflect the level of adulteration or handling of the drug. Adulteration by sand or earth is immediately detected as the total ash is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica. Therefore, the low values of total ash and acid insoluble ash obtained in this study indicate high level purity.

Knowledge of the pH of an excipient is an important parameter in determining its suitability in formulations since the stability and physiological activity of most preparations depends on pH. 1% w/v solution of TBG polysaccharide in water gave a pH of 6.36. The neutral pH of TBG polysaccharide implies that when used in uncoated tablets, it may be less irritating to the gastrointestinal tract. It may find useful application in formulation of acidic, basic and neutral drugs.

The bulk and tapped density gave an insight on the packaging and arrangement of the particles and the compaction profiles of the material. Compressibility value up to 15% usually results in good to excellent flow properties and indicates desirable packing characteristics. Compressibility index above 25% are often sources of poor tabletting qualities. Between these two value less than optimum performance might be anticipated and require modification of the formulation during process development. Results in the table 4 of TBG would be accepted to have the moderate flow and compressibility property.

Kawakita plot is used to analyze the behavior of powder from the bulk density state to the tap density state. The constants 'a' and 'b' of Kawakita plot were determined from the slope and intercept of graph of n/c versus number of tapping. The value of 'an' indicated compressibility or densification due to tapping and 'b' as rate of achieving final packing. The high value of 'a' and small value of 'b' indicated poor flowability and higher cohesiveness. Hence, from the results of Kawakita plot,

TBG polysaccharide powder had a higher compactability. According to the published results, the compactability and cohesiveness values obtained indicate fair flow ability and moderate cohesiveness.<sup>23, 24</sup> The flow behavior and the effect of ageing on the viscosity of a 1.0 % w/v aqueous dispersion of the polysaccharide powder in different fresh solutions (water, buffer and 0.1 N Hcl.) are shown in Figure 1. The viscosity of the polysaccharide dispersion decreases with an increase in shear rate. This is indicative of pseudo plastic or shear thinning behavior. At high shear rates, the decrease in viscosity can be attributed to a decreasing number of chain entanglements.<sup>25</sup> There is no significant difference in the viscosity after the colloidal solution of the polysaccharide powders were stored in humidity chamber for 90 days.

The microbial quality of the polysaccharide was assessed to determine their acceptability for use as pharmaceutical excipient in oral formulations. The total viable aerobic count and fungal count of the TBG polysaccharide was lower than that of the prescribed limit of European pharmacopoeia. The purified polysaccharide did not contain *E. coli*, *Pseudomonas aeruginosa*, *Salmonella spp.* and pathogenic Staphylococci. Microbial quality of purified polysaccharide was satisfactory and met the European Pharmacopoeia.<sup>18</sup> Analysis of elemental (C, H, N) composition of the purified polysaccharide indicated C, H, and N contents to be 39.31, 5.56 and 0.18 (w/w %) respectively.<sup>26</sup>

Scanning electron micrographs of TBG polysaccharide are shown in Figure 2. The micrographs of the TBG polysaccharide provide the surface morphology of the polysaccharide. The particles are mostly seen in aggregates of irregular shapes and dimensions which are fibrous in nature. The shape and structure or surface topology of the polysaccharide may be affected by the method of extraction and purification or preparation of the product.<sup>27</sup>

Differential scanning calorimetry (DSC) was used to measure the occurrence of exothermal or endothermal changes with an increase in temperature. Figure 3 shows the DSC curve of the polysaccharide. Glass transition temperature 49.8°C and there was no melting peak. The lower value was thought to be due to plasticization caused by residual water molecules remaining unmoved during fast scanning. The continuous endothermic transition is indicative of moisture loss in the sample. The weight loss onset (representing the onset of oxidation or decomposition) of 240°C suggests that the polysaccharide has good thermal stability. The onset peak and conclusion temperature of phase transition were observed to be 112.25°C. Structural and functional group differences in polysaccharides influence the thermal behavior and affect the transition temperature. The result

implies that TBG polysaccharide may structurally be stable and good thermal stability. The X-ray diffraction pattern of TBG polysaccharide is shown in Figure 4. The sample shows peaks at approximately 22°, 42° and 48° 2θ. However, other peaks are very weak and unresolved or are shoulders on more intense peaks. The result of the XRD confirms that of the DSC which shows that, TBG exhibits both crystalline and amorphous portion.<sup>28</sup>

The IR spectrum is shown in Figure 5. Peaks at 969.40 and 831.75 are due to finger print regions for carbohydrates, the absorption peaks at 1604.43 cm<sup>-1</sup> and 1412.15 cm<sup>-1</sup> are indicative of acetyl group. The absence of significant aromatic stretches in the 1600-1690 cm<sup>-1</sup> region and the weakness of the stretches imply that there is modest amount of cross linking by peptides. The sharp bands at 2925.19 cm<sup>-1</sup> is characteristic of methyl C-H stretching associated with aromatic rings. Peak at 3264.28 cm<sup>-1</sup> is due to hydrogen bonded hydroxyl groups that contribute to the complex vibrational stretches associated with free inter and intra molecular bound hydroxyl groups

which make up the gross structure of carbohydrates.<sup>29</sup> This is all consistent with a polysaccharide structure that is neither starch nor cellulose, but has some peptide cross links and some amino sugars.

### Conclusion

The results obtained in this study were established for the first time regarding the physicochemical & structural characteristics of the polysaccharide from *Terminalia belarica* gum (TBG). Studies indicated that the purified polysaccharide with such properties can therefore be used as polymer in novel drug delivery system to prolong the drug release. The high thermal stability of the polysaccharide indicates that it can be used as pharmaceutical excipient even under conditions of high thermal stress. The relative abundance and easy availability of TBG polysaccharide may allow it to serve as an alternative to presently available pharmaceutical excipients.

**Table 1. Physicochemical properties of purified polysaccharide of *Terminalia belarica* Gum**

Parameters		Results (Mean ± SD)				
Swelling Index (% v/v)	Water	270 ± 5.5				
	0.1N HCL	295 ± 8.06				
	Phosp hate buffer (pH7. 4)	310 ± 14.17				
Loss on Drying (% w/w)		3.96 ± 0.01				
Total ash (% w/w)		5.8 ± 0.08				
Acid insoluble ash (% w/w)		0.08 ± 0.03				
Ph		6.3 ± 0.01				
Angle of repose (degree)		23				
Bulk density (g/ml)		0.59 ± 0.06				
Tapped density (g/ml)		0.86 ± 0.04				
Compressibility index (%)		17.66				
Hausner ratio		1.46 ± 0.21				
<b>Kawakita plot</b>		<b>Slop e</b>	<b>a</b>	<b>b</b>	<b>r<sup>2</sup></b>	<b>1/b</b>
		3.0 69	0.325	0.0191	0.99 8	82. 54
Microbial quality		1.22 ± 0.21				
Total viable aerobic count (cfu/gm)		0.29 × 10 <sup>3</sup>				
Total fungi count		< 100				
<i>Escherichia coli</i>		Absent				
<i>Staphylococcus aureus</i>		Absent				
<i>Pseudomonas aeruginosa</i>		Absent				
<i>Salmonella Spp</i>		Absent				

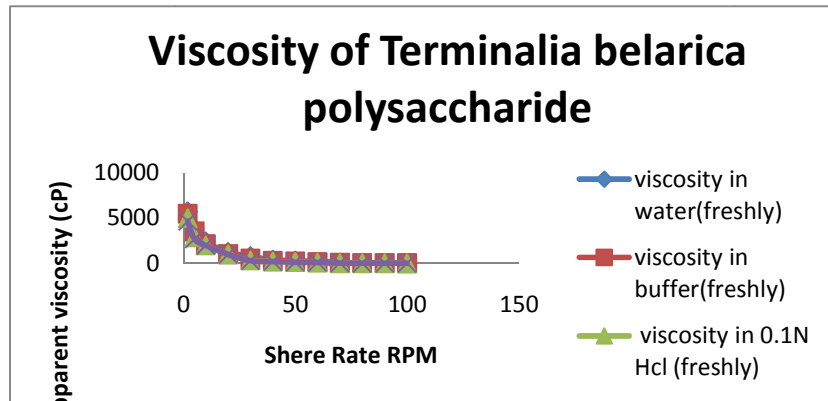


Figure 1: Flow behavior of a 1% w/v aqueous dispersion of TBG polysaccharide powder in different solution at room temperature and the effect of ageing on viscosity (n=3, mean  $\pm$  SD)

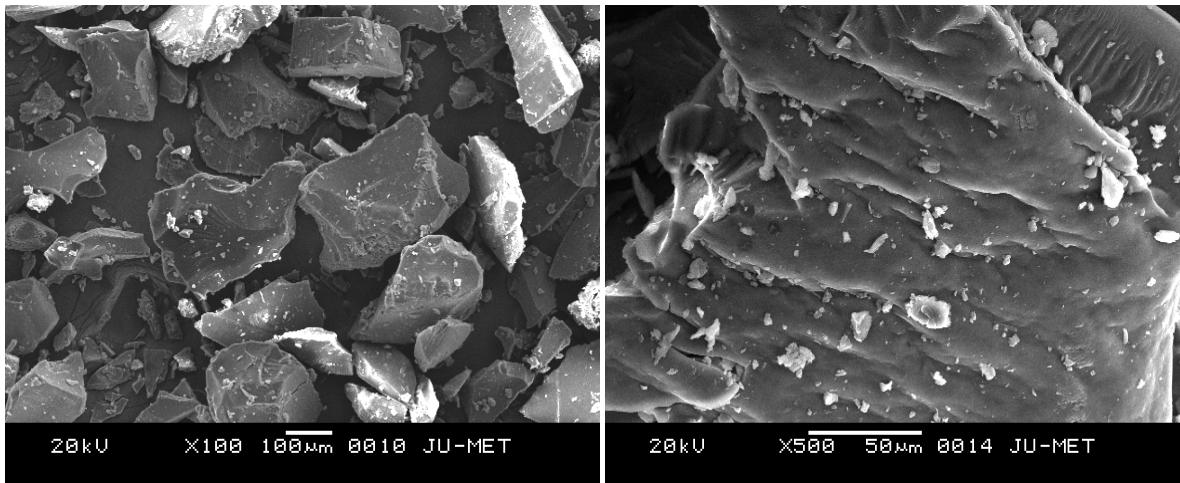


Figure 2: Photomicrographs as recorded by scanning electron microscopy of TBG (magnification 100X and 500X, Scale bar 100  $\mu$ m, 50  $\mu$ m)

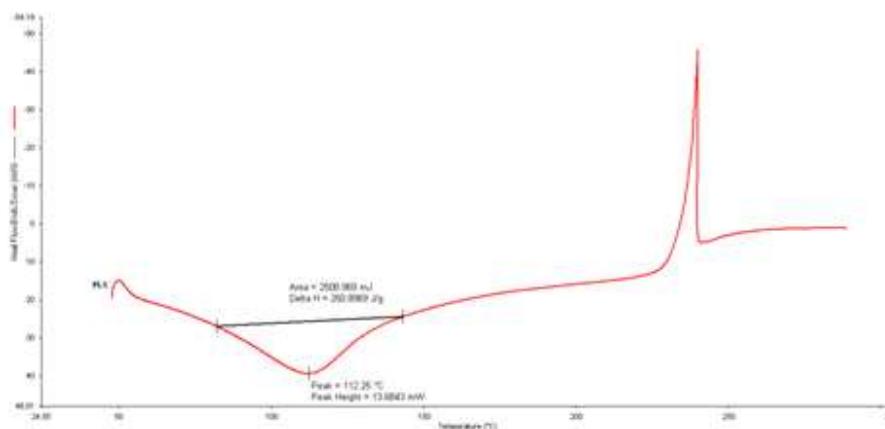


Figure 3: DSC characterization of TBG polysaccharide

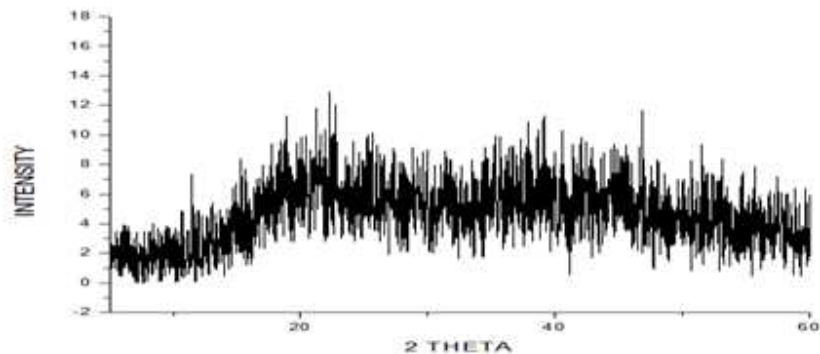


Figure 4: X-ray diffraction pattern of TBG polysaccharide

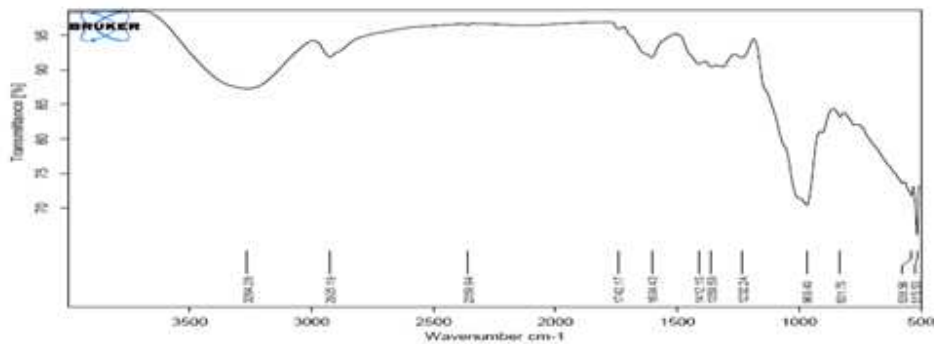


Figure 5: FT-IR spectrum of TBG polysaccharide, indicating the various peaks

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