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Research paper

Structural elucidation of galactomannan from ‘Petai Belalang’(*Leucaena leucocephala*) and its modified analogue

Shazlinda Shirajuddin[1], Dzaraini Kamarun[1]*, Sabu Thomas[2]

[1]*Orchestrated Polymer Research Group (OPoR)*
Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]*International and Inter University Centre for Nanoscience and Nanotechnology*
Mahatma Gandhi University, 686560 Kottayam, Kerala, India
**Corresponding author E-mail: dzaraini@salam.uitm.edu.my*

Abstract

Galactomannan (GM) is a biomoleculer polymer present as a storage carbohydrate in endosperm of many leguminous plant and fungal cell wall. Commercial GMs were obtained from plants such as guar gum (Cyamoposis tetragonolobus), locust beans (Ceratonia siliqua) and tara gum (Caesalpinia spinosa). In this project GM was extracted from seeds of a local plant ‘petai belalang’ scientifically known as Leucaena leucocephala. The GM was further conjugated with acryloyl groups for further crosslinking reactions. We report here the structural properties of the modified analogue of GM based on several spectroscopic techniques such as FTIR, NMR and XRD. The morphology of the galactomannan analogue (aGM) was identified from SEM images at several magnifications. We also included discussions on comparisons of the aGM from its parent structure. GM was successfully conjugated with the acryloyl group as verified by the presence of vinyl stretching bands and vinyl protons in the FTIR and H_1 NMR spectrum of aGM respectively. This is supported with SEM images that show distribution of granules along the aGM surfaces as compared to a smooth surface of its parent compound. The successfulness of the conjugation process was further verified from data obtained from thermogravimetric analysis (TGA).

Keywords: *conjugated, galactomannan, leguminous plant, morphology, spectroscopic.*

1. Introduction

Galactomannan (GM) is a biomolecular polymer that consists of long chain of β-(1-4) mannan unit with attached D-galactose (G) branches linked via α-(1-6) linkages distributed along the mannose chain. This hydrophilic polysaccharide can be obtained from leguminous plant endosperm as a storage carbohydrate and in microbes such as fungal cell wall. 70 species from Leguminosae family have been identified to contain GM [1]. Commercial GMs were obtained typically from guar gum (Cyamoposis tetragonolobus), locust beans gum (Ceratonia siliqua) and tara gum (Caesalpinia spinosa). Extractions and characterization of other sources of GMs have also been reported [2–5].

GM are commercially known as gums in food and pharmaceutical industry; are safely consumed as food additives or drug carrier. Enzymes that are present in the human intestine degrade GM by attacking specific sites in its structure [6]. GM are widely used in the food industry as stabilizers, thickeners and binders in dairy products, frozen foods, and dressings in order to improve the food texture and shelf life [7]. Besides that, GM are biodegradable material that also used as edible films or coatings for the food products [8]. In pharmaceutical industry, GM are used as a drug carrier for colonic specific drug delivery. The present of microbial in the human intestine that able to degrade GM structure allow drug to be release at specific targeted site [7, 9].

GM extracted from variable sources show variations in mannose/galactose ratio, substitutions, fine structure and molecular weight [1, 7, 10]. Moreover, the properties are also influenced by the extraction and purification techniques used [11]. In its native form GM shows limited characteristics and need to be modified to expand its versatility. Several chemical modifications such as carboxymethylation, carbomoyethylation, sulfation, and phosphorylation were tried with GM molecules to improve its chemical properties and biological functions [6].

Carboxymethylation and carbomoyethylation were carried out to improve the solubility of GM [12–15].High swelling characteristic of galactomannan can cause the drug carrier matrix to burst before delivering drug. Phosphorylation can control high swelling characteristic of GM purposely for colon drug delivery (control burst release effect) [6] and also antioxidant properties [16]. Sulfation reaction can synthesized sulfated analogue of galactomannan that mainly used for antiviral and anticoagulant properties [17].

Despite hydrophilic characteristic of GM, variation of GM molecular structure has significant effect on the solubility, viscosity and interaction between GM with other polysaccharides [1]. Intermolecular forces through abundance of hydroxyl group in the GM structure cause entanglement of the polymer and effect solubility in the water [6]. In this study GM was modified to increase its solubility and chemical reactivity for further reaction in its application as a drug carrier. The modified GM analogue was compared with unmodified GM in terms of its thermal stability and morphology.

2. Experimental

2.1. Conjugation of galactomannan with acryloyl group

Extraction of GM from petai belalang was reported elsewhere [18]. Acrylated galactomannan (aGM) was prepared by conjugating GM with acryloyl chloride. In brief the procedure was as follows:

4 ml trimethylamine and 2 ml methyl ethyl ketone was added into an aqueous solution of GM solution in the presence of hydroquinone as an inhibitor. The GM solution was then added dropwise into a solution of acryloyl chloride in methyl ethyl ketone in a three-necked round bottom flask under nitrogen environment at 0-5 ^{o}C. The mixture was left under stirring for 24 hours at room temperature to ensure completion of reaction. Acetone was then added into the GM and acryloyl chloride mixture to precipitate aGM compound. aGM produced were purified through dialysis against pure water using a dialysis membrane with MWCO of 3500 for 12 hours. The lyophilized form of aGM was obtained after the dialyzed product was freeze dried.

2.2. Structural elucidation of acrylated galactomannan (aGM)

Structural characterization of aGM was determined by using Perkin Elmer Spectrum 100 FT-IR Spectrometer. Transmission sampling was carried out using KBr pellet and sample were analysed in the range 4000 cm^{-1} - 450 cm^{-1}. H_1 proton analysis was carried out on Varian Mercury 400 NMR spectrometer. The crystallinity of aGM was determined from X-ray diffraction analysis, XRD using Bruker X-ray diffractometer with angle range of 10-80^{o}.

2.3. Thermal stability and fractions present in galactomannan and acrylated galactomannan (aGM) by using thermogravimetry (TGA)

Thermal stability of GM and its analogue were identified using DTA-TG apparatus over a temperature range of 20-1000 ^{o}C. The various fractions present before and after the modifications will indicate whether the conjugation process has proceed.

2.4. Morphology study

The morphology of GM and its modified analogue were studied by using scanning electron microscope (SEM, JSM-640, JEOL). The surfaces of the sample were coated with thin layer of gold of about 100 Å and were analyzed at an accelerating voltage of 15kV.

3. Results and Discussion

3.1. Spectroscopic identification of acryloylated galactomannan (aGM)

Fig. 1 shows the FTIR spectra of GM and aGM. Two characteristics peak at 3400 and 2925 cm^{-1} were assigned to hydroxyl (-OH) stretching vibration and asymmetric C-H functional groups of carbohydrate ring respectively. Bending vibration of C-H at 815 and 870 cm^{-1} indicate (α and β conformer) of glycosidic linkages, α-D- galactopyranose units and β-B-mannopyranose units respectively. The peak at 1155 cm^{-1} represent C-O bond of the ring while peak at 1079 cm-1 and 1025 cm^{-1} represent C-O bond of C-OH bending [4, 18, 19]. The absorption band at 1642 cm^{-1} represent the bending vibration of water molecules present in the sample [20].

No major differences were observed in the 2 spectra except for the presence of a new peak at 1558 cm-1 in aGM spectrum which corresponds to C=C stretching of a vinyl group attached to the parent structure upon conjugation with acryloyl group.

Each of sugar unit in GM contain primary hydroxyl at C_6 and secondary hydroxyl at C_2 and C_3 for the modification to take place [6, 14]. However modifications took place more easily at the primary hydroxyl group of C_6 due to higher reactivity and lesser steric hindrance at C_6 of galactomannan [6, 14].

NMR spectrum of aGM in Fig. 2 shows signals of H_1 in the range of 5.535 to 6.062 ppm which correspond to protons of the vinyl group (–CH=CH2) of acryloyl chloride [21, 22]. This result confirmed the conjugation of GM with acryloyl chloride to form aGM molecule. Modification of GM with acryloyl group helps improved its solubility in water. aGM was found to dissolve completely in distilled water unlike its parent molecule which showed limited solubility in the same solvent.

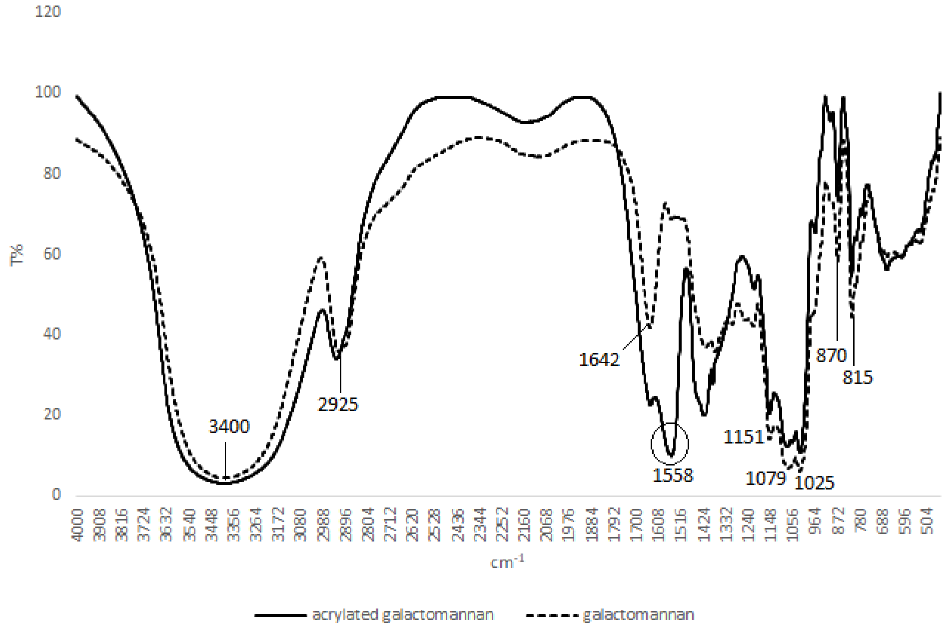

Fig. 1: FTIR spectrum of galactomannan and acrylated galactomannan

The XRD pattern of GM and aGM are presented in Fig. 3. GM shows broad peak between 16° to 29° with low intensity indicating the amorphous nature of galactomannan. The same amorphous nature of GM was shown by galactomannan extracted from *Dichrostachys cinerea* seeds [23]. The amorphous nature of galactomannan was not affected by the modification reaction as seen from the same broad and low peak of the XRD pattern in the same region.

3.2. TGA of GM and its modified analogue

Table 1 summarized the degradation temperatures of the various polymeric fractions present in GM and aGM generated from the TGA curves of the two compounds as shown in Fig. 4.

Based on the TGA curve of both polymer the initial degradation at 70 °C to 100 °C was due to the loss of volatiles such as water molecules. Both polymers consisted of 2 polymeric fractions as shown by their onset degradation peaks at temperatures 270 °C and 348 °C for GM correspond as galactomannan extracted from *Gleditsia triacanthos*, *Caesalpinia pulcherrima* and *Adenanthera pavonina* [19], while aGM degraded at 112 °C and 304 °C. However, the 2 polymeric fractions of GM were more thermally stable than those of aGM.

Table 1: Degradation temperature of fraction of galactomannan and acrylated galactomannan

Polymer	Fraction	Degradation Temperature	
		$T_{initial}$ (°C) (onset)	T_{final} (°C)
GM	1st	270	348
	2nd	348	552
AcGM	1st	112	304
	2nd	304	538

Furthermore, GM degraded completely after 552 °C with no residual mass. aGM degraded leaving a residual mass of about 25 % at temperatures higher than 538 °C. The residual mass left was assumed to be the acrylated form of aGM. The presence of residue as the third fraction in aGM suggest that the modification reaction has succeeded.

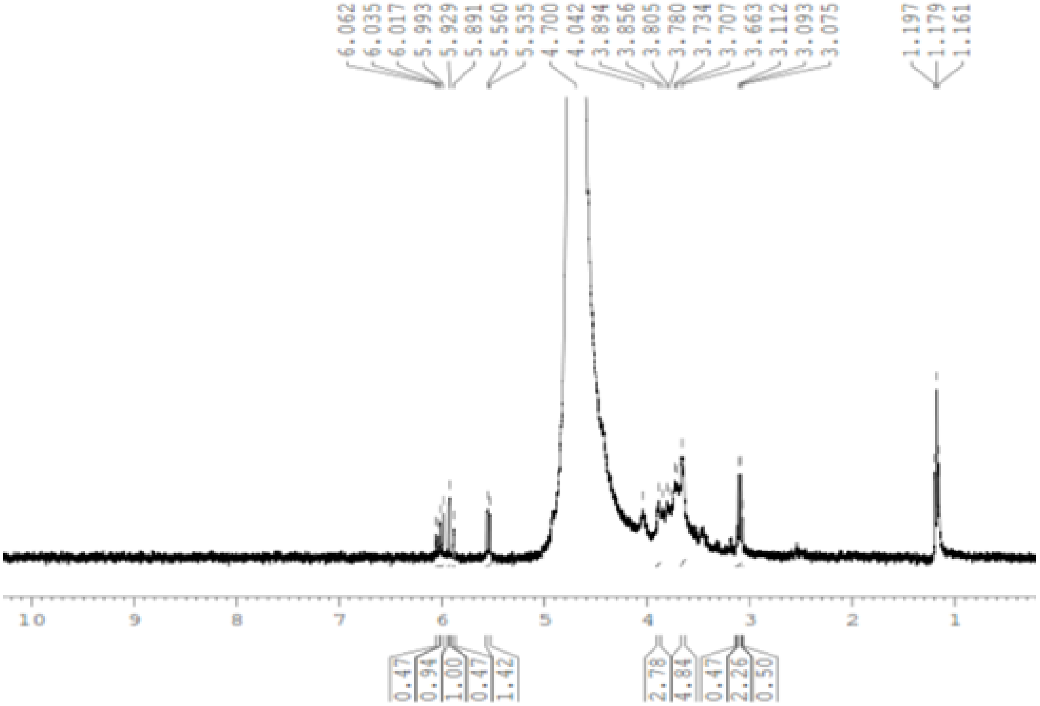

Fig. 2: H^1 NMR spectra of arylated galactomannan

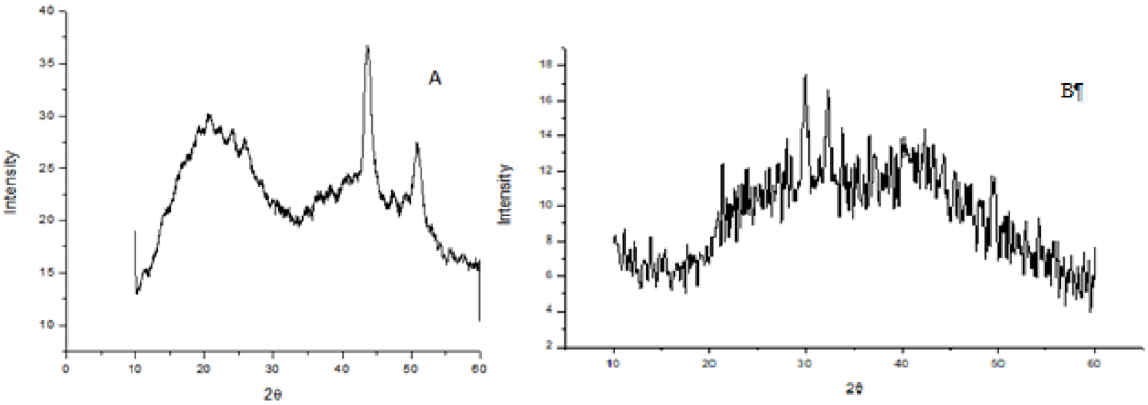

Fig. 3: X-ray diffraction of A) galactomannan and B) acrylated galactomannan

3.3. Morphology of galactomannan and acrylated galactomannan (aGM)

SEM images of GM and aGM taken at magnifications 500×, 1000× and 2000× are as shown in Fig. 5. The SEM images of Fig. 5 show clearly the different morphological surfaces of GM and aGM. The surfaces of GM are generally smooth with folded and tube-like structures; while aGM surfaces show nodule-like structures distributed along the tube-like and folded surfaces. The differences observed in aGM surfaces could be attributed to the conjugation of acryloyl groups onto GM molecules. Based on the SEM images, galactomannan long chain fibrous network seems like intertwined with each other as reported in the literature [24].

4. Conclusion

GM was successfully conjugated with the acryloyl group as verified by the presence of vinyl stretching bands and vinyl protons in the FTIR and H_1 NMR spectrum of aGM, respectively. The SEM images showed distribution of nodule-like structures along with the aGM surfaces as compared to a smooth texture of GM which suggest the possibility of successful conjugation of GM. Both GM and its modified analogue are amorphous in nature. However, the 2 polymeric fractions of GM were more thermally stable than those of aGM. The successfulness of the conjugation process was further verified by TGA data.

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