

Approaches to Modify the Nature of Xanthan Gum and Characterizations to Improve its Functionality

Abdul Nasir kurnool^{1*}, Ankit acharya¹, b. Ramesh²

¹Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, B.G-Nagara-571448, Karnataka

²Principal, Sri Adichunchanagiri College of Pharmacy, B.G-Nagara-571448, Karnataka

Abstract

Objective: In order to reduce the demerits of crude Xanthan gum, the present study was aimed to modify the nature of Xanthan gum by chemical and microwave irradiation approaches.

Materials and method: Synthesis of chemically grafted xanthan gum was carried out by the free radical co-polymerization technique using ceric ammonium nitrate as initiator. The morphological characteristics, structural composition, functional group and thermal behaviour of grafted gum were evaluated using SEM, FT-IR, XRD and DSC.

Results: FT-IR studies showed no change in vibrational frequencies of the functional groups in modified xanthan gum. Thermogram showed endothermic peak at 103.53°C and 124.31°C, represents amorphous nature of the gum. Surface morphology studies and X-ray diffractogram revealed that native xanthan was powdery and fibrous in nature, where as, grafted and irradiated gum were brittle in nature. The grafting process was optimized by varying the temperature, concentration of acryl amide and also by changing radiation frequency. Grafting efficiency was increased when irradiation time and concentration of acrylamide were optimum, further increase in irradiation time decreases the grafting efficiency. The viscosity of grafted and irradiated gum was comparatively lesser than that of crude xanthan gum, which might be due to break down of polysaccharide units into monomer units.

Conclusion: Thus it was concluded that the biodegradable and biocompatible grafted xanthan can be synthesized with high viscosity and higher grafting efficiency. In future such type of modified gum can gain attention towards pharmaceutical industry for the preparation of controlled and sustain release formulations.

Key words: Xanthan gum, co-polymerization technique, grafting efficiency, microwave irradiation

INTRODUCTION

For centuries man has made effective use of materials of natural origin in the medical and pharmaceutical field. Today, the whole world is increasingly interested in natural drugs and excipients [1]. In recent years, plant derived polymers have evoked tremendous interest due to their diverse pharmaceutical applications such as diluents, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository, they are also used in cosmetics, textiles, paints and paper-making [2,3].

Natural polymers are polymers which exist in natural and biocompatible those breaks down and lose their initial integrity with body components and should be non-toxic, capable of maintaining good mechanical integrity until degraded, and capable of controlled rates of degradation. A goal is not to illicit the immune response, and the products of degradation also need to be non-toxic. Disadvantages of natural polymers are microbial contamination, batch to batch variation, uncontrolled rate of hydration, reduced viscosity on storage [4]. Natural polymers were modified in different ways to obtain tailor-made materials to overcome such problems. Basically, two methods are available as the physical method and chemical method [5].

In physical method molecular interaction between polymers can be achieved by exposure to dry heat, saturated steam, microwave technology, UV and gamma radiation. Chemical method-polymers are treated with chemicals like aldehydes, epichlorhydrine, borax or glutaraldehyde. Temperature is one of the most favourable methods of cross-linking because it avoids both the application of harsh chemical materials for large-scale production and the diversity of equipment and methods used in their application [6].

Xanthan gum is a polysaccharide. When the xanthan gum is dissolved in aqueous solvent, its pH is around 7 and also exhibits pseudo plasticity behaviour. Xanthan gum possess number of monosaccharide subunits such as D-mannose, D-glucose, D-glucuronic acid and pyruvic acid [7]. Even though xanthan gum is having wide range of application in both food and pharmaceutical industries, it is also having some drawbacks such microbial attack, uncontrolled hydration, and drop in viscosity during storage, etc. So, in order to avoid such problems, present study was aimed to modify the nature of xanthan gum and characterizations to

improve its functionality and properties in the field of novel drug delivery systems [8].

MATERIALS AND METHODS

Material used: Pure Xanthan gum was procured from Techno Scientific Products, Bangalore, India. Ceric ammonium nitrate (CAN), acetone, ethyl alcohol and acrylamide were procured from Yarrow Chem. Pvt. All other chemicals used were of analytical grade. Millipore (MilliQ ®) water was used throughout the study.

Chemical modification of xanthan gum

The graft copolymer derived from acrylamide and xanthan gum was prepared by free radical induced method, where a specified amount of acrylamide was added to 30 ml of distilled water and stirred for half an hour. To the above mixture specified amount of Ceric ammonium nitrate (CAN) was added. Now this solution was added to aqueous dispersion of xanthan gum and stirred for about 2 h. The aqueous dispersion of xanthan gum was prepared by dissolving 1g of xanthan gum in 100 ml of distilled water under continuous stirring (table 1) [9].

Table 1: Synthetic details of acrylamide-grafted and microwave irradiated xanthan gum

Batch code	Xanthan gum (gm)	CAN (mg)	Acrylamide (gm)	Irradiation time (min)
XG-1	1	200	4	--
XG-2	1	200	4	2
XG-3	1	200	4	4
XG-4	1	200	4	6
XG-5	1	200	6	--
XG-6	1	200	6	2
XG-7	1	200	6	4
XG-8	1	200	6	6
XG-9	1	200	8	--
XG-10	1	200	8	2
XG-11	1	200	8	4
XG-12	1	200	8	6

Microwave irradiation of xanthan gum

The entire procedure for this method was same as that of grafting method, but sample was further irradiated by micro-wave (LG micro-wave oven) at 750 W frequency and time periods. The micro-wave irradiated sample was then left for some period of time for cooling and precipitated using 4:1 ratio of acetone: ethanol and filtered. The precipitate was further washed with 30% aqueous ethanol to remove unreacted homopolymer and other reagents. The precipitated so obtained was dried at 45°C to a constant weight and converted to fines. Now the obtained fine was regarded as grafted xanthan gum. Finally, grafted xanthan gum was optimized based up on concentrations of reactants and irradiation temperature in order to get highest % of grafting copolymerization [10].

The % grafting (% G), grafting efficiency (%GE) and % conversion (%C) was calculated using the equation followed by Santanu Kaity *et al.* 2013.

Characterization studies

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was performed to characterize the presence of different functional groups and the molecular structure of the crude, chemically grafted and micro-wave irradiated Xanthan gum. Samples were prepared by press pellet technique. Shimadzu, FTIR-8400S, IR spectrophotometer was used during this research work [9].

Thermal analysis

Thermal properties of the crude, chemically grafted and micro-wave irradiated xanthan gum were analyzed by Differential Scanning Calorimeter (Shimadzu DSC-60, Shimadzu Limited Japan). The samples were heated in a thermetically sealed aluminium pans. Heat runs for 3 gm of sample was set from 10 °C to 350°C at a heating rate of 10°C/min, using nitrogen as blanket gas [10].

Scanning electron microscopy (SEM)

The surface morphology of the xanthan gum, chemically modified (grafted) xanthan gum and micro-wave irradiated xanthan gum was examined using a SEM (Quanta 250 FEG Model No. 1027641, Czech Republic). The samples were gold coated to increase the conductivity of the electron beam. An accelerating voltage of 10 kV and a working distance of 12 mm at spot size of 45 were used [9,10].

X-ray diffractometry analysis (XRD)

XRD analysis of crude gum, chemically grafted gum and micro-wave irradiated xanthan gum were analysed using X-ray diffractometer (Bruker AXS D8 Advance, Bruker, Germany, configuration: Vertical, Theta/2 Theta geometry). The X-ray source was Cu, with wavelength 1.5406 Å and Si (Li) PSD detector employed. The diffractometer was run at a scanning speed of 2°/min, a chart speed of 2°/2 cm per 2θ and an angular range fixed between 3° and 80° [9].

Determination of water uptake of crude and modified xanthan gum

A sample of 1 g of crude and modified xanthan gum was immersed in equal volume of distilled water at 37 °C for 72 h. The weight of the swollen samples was determined after removal of the surface liquid with tissue paper. The water uptake was then calculated according to the following equation.

$$W_u = [(W_f - W_i) / W_i] \times 100$$

where W_u , W_f and W_i are water uptake, final weight and initial weight of the sample, respectively [9, 10].

Viscosity measurement

The viscosity of crude xanthan gum and modified xanthan gum (0.2%, w/v) was determined by a Brookfield digital viscometer

(Model LVDV-E, USA) at 32.7 °C. The samples were dissolved in hot water and rotated at 0.5 rpm using spindle (Spindle no.T-96). Another study was also conducted to determine the change in viscosity of the crude and modified xanthan gum by storing them into well closed glass bottle for the period of four months (at room temperature)[8].

RESULTS AND DISCUSSION

Synthesis of chemically modified xanthan gum (Am-g-xanthan gum)

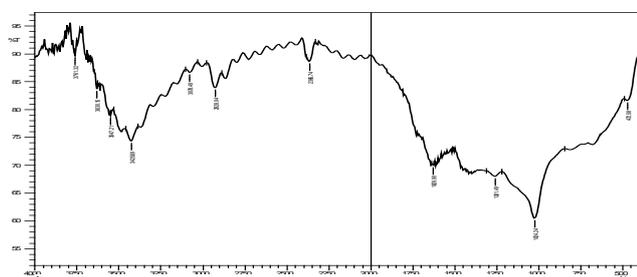
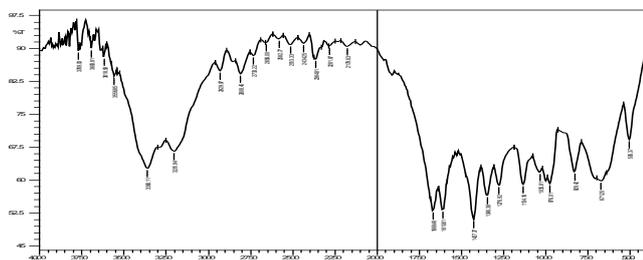
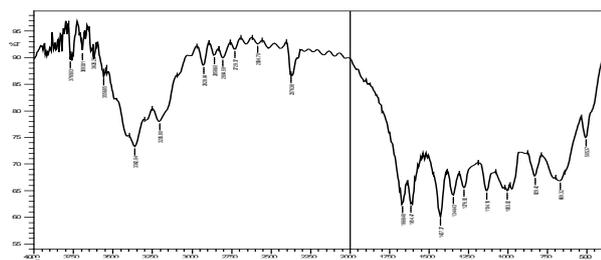
Synthesis of chemically grafted xanthan gum was carried out by the co-polymerization technique, where the acrylamide form linkage with the backbone of xanthan gum through free radical formation mechanism. During this grafting, free radical graft copolymerization reaction was initiated by the ceric ammonium nitrate. Acrylamide was used as reactant, as it favoured the grafting reactions with xanthan gum. In an initial step ceric ion attacks the xanthan gum and forms a xanthan-ceric complex (IV). The ceric ion complexes are then reduced to ceric (III) ions by the oxidising hydrogen atom and thereby formed a free radical onto xanthan gum backbone. The grafting of acrylamide onto xanthan gum occurs when the free radicals formed reacted with monomer unit via covalent bond and finally formed grafted xanthan gum. During this process propagation reaction between acrylamide and xanthan gum takes place. Finally, termination was achieved through a combination of two radicals. The grafted sample was further subjected to micro-wave irradiation for specified period of time in order to induce the reaction. Table 2 showed that the % grafting was increased in those samples which were exposed to the micro-wave irradiation (750W), where micro-wave acts as catalyst. The % grafting was increased up to 4 minutes of exposure and then the rate of grafting was decreased significantly. The grafting rate was decreased up on prolonging the exposure time of irradiation, which might be due to degradation of the xanthan gum backbone (frequent chain breakage) and resulted in the homo-polymer formation. Optimized concentration of acrylamide and irradiation exposure time minimized the formation of homo-polymers and resulted in the generation of free radical sites on the back bone of xanthan gum which might be due to splitting of polar functional groups present on the backbone of the xanthan gum. As xanthan is a polysaccharide molecule, it contains water molecule in its structure. These water molecules absorbs micro-wave and produces heat, there by increases the hydrophobicity. Because of this reason micro-wave irradiation of grafted xanthan gum showed greater hydrophobicity and lower viscosity in aqueous solution compare to crude xanthan gum. Initially grafted gum was extracted using acetone alone, but the percentage of extraction was very low. Then the extraction solvent was optimized and 4:1 ratio of acetone: ethanol was used as extraction solvent. Therefore, it may be concluded that mixture of acetone: ethanol (4:1 ratio) can successful to remove the homopolymer formed during the polymerization reaction. Among all synthetic batches, batch code XG-11 showed highest grafting efficiency which was comperatively higher than XG-12, though the irradiation time was longer for XG-12 and others parameters were same as XG-11. This may be due to the fact that, in case of XG-12 exposure time was longer and which in turn leads to breakage of propagated chains on the free radical sites. Batch XG-1 and XG-5 did not showed any sign of grafting, as these batches are not micro-wave irradiated. From the result obtained it was clear that micro-wave irradiation plays important role in polymerisation process. In conclusion, optimized concentration of acrylamide and micro-wave irradiation time leads to increase the grafting efficiency of xanthan gum.

Table 2: Results of grafting efficiency (%GE), % grafting (%G) and % conversion (%C)

Batch code	Weight after grafting (gm)	%G	%GE	% C
XG-1	0.45	--	--	--
XG-2	1.28	28	14	32
XG-3	2.10	110	27.5	52.5
XG-4	1.805	80.5	20.125	45.1
XG-5	0.708	--	--	--
XG-6	1.87	87	14.5	31.16
XG-7	3.308	230.8	38.46	55.13
XG-8	2.69	169	28.166	49.33
XG-9	1.14	14	1.75	14.25
XG-10	2.005	100.5	12.56	25.06
XG-11	5.604	460.4	57.55	70.05
XG-12	3.066	200.6	25.82	38.32

Fourier transform infrared spectroscopy

Results of FTIR spectrum of crude, chemical modification and micro-wave irradiation xanthan gum are shown in figure 1-3. The spectrum of crude xanthan gum exhibits a prominent broad band at 3547.21 cm^{-1} , a typical stretch for the OH groups and same hydrogen-bonded OH groups are responsible for interacting with water molecules. The peaks corresponding to C-H stretching vibrations of the $-\text{CH}_2$ groups in the xanthan gum occurs at 2929.97 cm^{-1} . The peaks at 1670.41 cm^{-1} and 1629.90 cm^{-1} are due to stretching of the carbonyl (C=O) esters of acetyl groups and a symmetrical stretching of (C=O) for the carboxylate group, respectively. Another band at 1427.37 cm^{-1} , can be ascribed to the angular deformation of CH_3 . Absorption bands at 1024.24 cm^{-1} may be due to stretching of the C-O bond of the glycosidic bonds. Same bands are present on the modified gum.

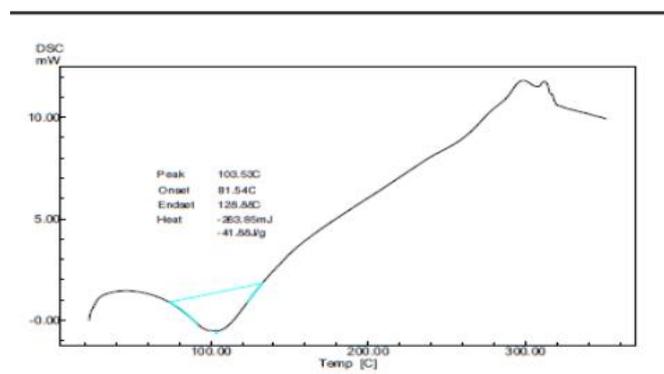
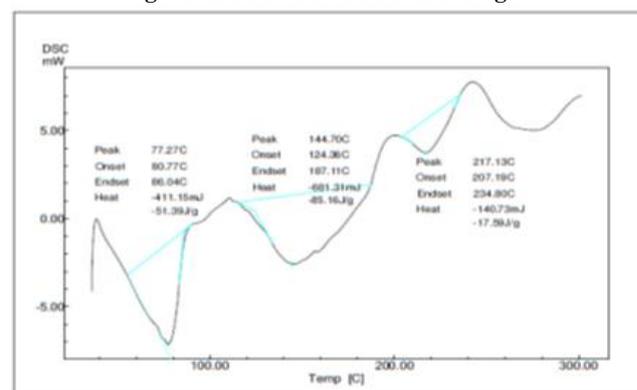

Fig. 1: Infrared spectra for crude xanthan gum

Fig. 2: Infrared spectra for grafted xanthan gum

Fig. 3: Infrared spectra for irradiated xanthan gum

Thermal analysis

Thermal characterization by diffraction scanning calorimetry (DSC) was employed to investigate the physical forms, thermal transition, melting behaviour and chemical and stability properties. Presence of endothermic peak at 103.53°C represents that analysed Xanthan gum was amorphous nature. Chemically modified (grafted) Xanthan gum has four endothermic peaks. First broad endothermic peak was observed at 63.62°C , which might be loss of moisture present in the sample. Second endothermic peak was observed at 124.31°C , corresponding to its enthalpy of fusion (ΔH_f) at -69.73 J/g . However, grafted Xanthan gum showed slightly increase in enthalpy value compared to its crude form. Fourth peak at 272.01°C (endothermic peak) was for polyacrylamide, which may result from ammonia loss and imine formation. During interpretation of DSC curves of microwave irradiated Xanthan gum, altogether three endothermic peaks were obtained at 77.27°C , 144.70°C and 217.13°C for first, second and third endothermic peak respectively. The profile of irradiated Xanthan gum was similar to that of the chemically grafted Xanthan gum; however, endothermic peaks related to the polyacrylamide degradation were displaced to lower temperatures of 217.13°C . T_p value for grafted gum and irradiated gum was higher than that of the crude gum, higher the T_p value, the more thermodynamically stable is the macromolecule, which might be due to the linkage between polymer chain and acryl amide side chains. Results are showed in table 3 and figure 4-6.

Table 3: Some important DSC parameters of tested samples

Sample name	Peak ($^\circ\text{C}$)	Onset ($^\circ\text{C}$)	Endset ($^\circ\text{C}$)	Enthalpy (J/g)
Crude Xanthan	103.53	81.54	128.88	- 41.85
Grafted Xanthan	144.70	124.36	187.11	- 85.16
Irradiated Xanthan	124.31	109.07	151.40	- 69.73


Fig. 4: DSC curve of crude xanthan gum

Fig. 5: DSC curve of chemically modified (grafted) xanthan gum

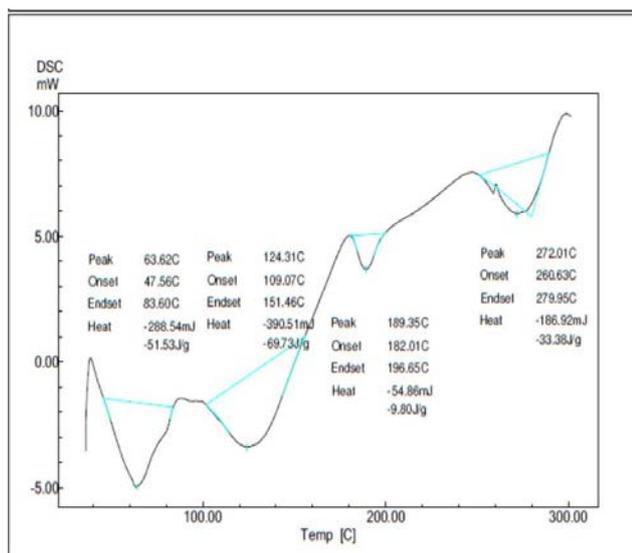


Fig. 6: DSC curve of micro-wave irradiated xanthan gum

Scanning Electron Microscopy analysis (SEM)

Surface morphology of crude, chemically grafted and micro-wave grafted Xanthan gum was investigated using scanning electron microscopy. SEM (Quanta 250 FEG Model No. 1027641, Czech Republic) model was used and each sample was analyzed under three different magnifications i.e. X 400, X 1000, X 4000 (figure 7). The SEM image of crude xanthan gum was found to be more powdery and fibrous in nature. Fibers of the xanthan gum seemed to be present in associated form i.e. cross linked. When the SEM images of acrylamide grafted xanthan gum was observed, the grafted gum seemed to be lumpy (mass like) with undulant sharp breaking points (figure 7 D, E and F). These sharp breaking points indicated that the chemically modified xanthan gum was brittle in nature. The surface morphology of micro-wave irradiated xanthan gum (figure 7 G, H and I) was same as that of the chemically grafted xanthan gum, but the lumpy mass formed by irradiated xanthan gum was smaller than chemically modified form. The granules size of the grafted gum and micro-wave irradiated xanthan gum was comparatively larger than that of the crude xanthan gum.

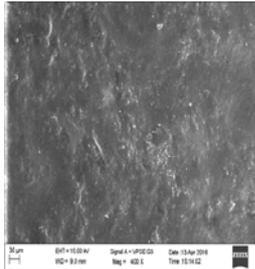
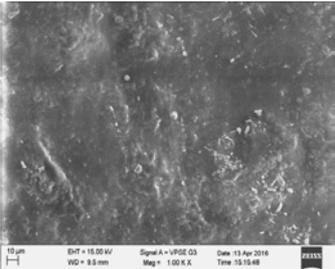
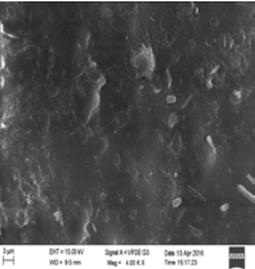
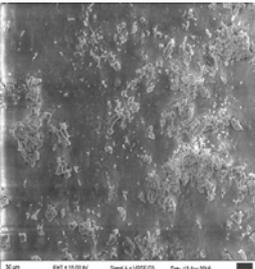
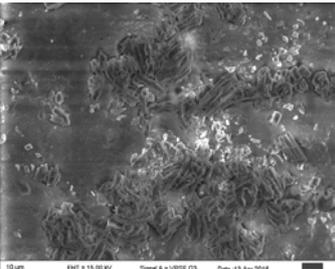
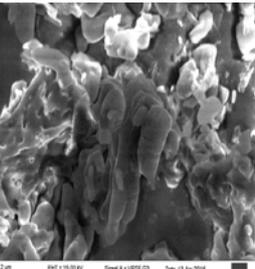
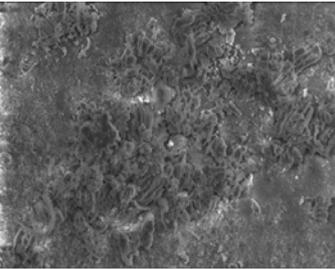
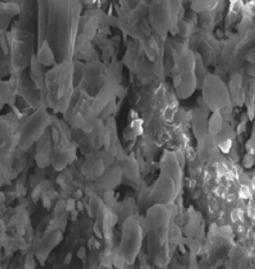
SEM images of various samples		
Magnification		
X 400	X 1000	X 4000
 A	 B	 C
 D	 E	 F
 G	 H	 I

Fig. 7: SEM images of crude (A=X 400, B= X1000, C= X 4000), chemically modified (grafted) (D= X 400, E= X 1000, F= X 4000) and irradiated xanthan gum (G= X 400, H= X1000, I= X 4000)

X-ray diffractometry analysis (XRD)

X-ray diffractogram of crude, chemically grafted and micro-wave irradiated Xanthan gum were represented in Figure. 8-10. X-ray diffractometer (Bruker AXS D8 Advance, Bruker, Germany, was used during the study. X-ray diffractometry analysis was carried out to determine the nature (crystallinity or amorphous) of the sample. Figure 8 showed that diffractogram of crude Xanthan gum was found to be largely amorphous. In the diffractogram of crude Xanthan gum, there was only one crystalline peak at 2θ value of 22.30° with the relative intensity of 60.23%. The diffractogram shown in figure 9 showed that chemically modified Xanthan gum increases the crystallinity of the native Xanthan gum. This can be proven by fact that the grafted Xanthan gum displayed nineteen visible and distinguishable diffraction peaks at 2θ values in the range of 11.873° , 18.17° , 19.29° , 20.59° , 23.0° , 23.94° , 24.54° , 26.08° , 27.28° , 28.43° , 31.39° , 31.64° , 32.98° , 36.16° , 45.30° , 45.50° , 56.24° , 66.16° and 75.18° . The peaks exhibited by grafted gum were comparatively higher when compared to single peak obtained by the crude gum. This result verifies that the maximum grafting was occurred when acrylamide reacted with crude Xanthan gum and resulted end product was found to be polyacrylamide grafted Xanthan gum. The increase in crystallinity of grafted gum could be attributed to the contribution made by acrylamide. When grafted xanthan gum was further treated with micro-wave irradiation the crystallinity of Xanthan gum was significantly increased. The micro-wave irradiated gum displayed visible and highly distinguishable diffraction peaks (figure 10) at 2θ values in the range of 11.90° , 18.22° , 19.31° , 20.58° , 22.93° , 23.91° , 24.49° , 26.04° , 27.21° , 28.47° , 31.57° , 32.94° , 34.18° , 36.06° , 45.45° , 47.63° , 48.29° , 49.18° , 53.77° , 56.39° , 65.97° and 75.35° . An increase in crystallinity following micro-wave irradiation of grafted Xanthan gum was also detected by Anjum *et al.* (2015) and Singh *et al.* (2009).

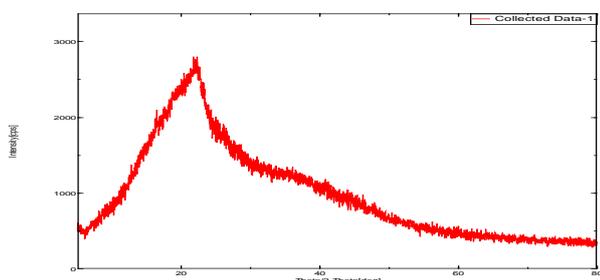


Fig. 8: XRD of crude Xanthan gum

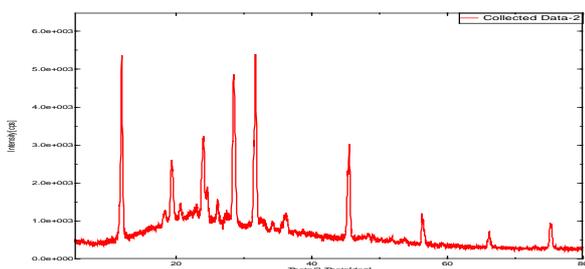


Fig. 9: XRD of grafted Xanthan gum

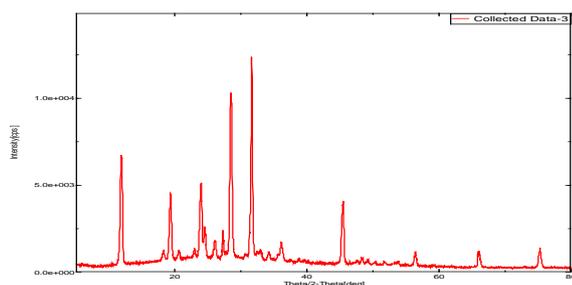


Fig. 10: XRD of irradiated Xanthan gum

Determination of water uptake of crude and modified xanthan gum

Water uptake capacity of crude and modified xanthan gum was determined at 37°C after equilibration in distilled water. The preliminary tests lead to the equilibrium time required for complete swelling and was shorter than 60 h. Therefore, the experiments were carried out considering the water uptake calculation after equilibration in distilled water for 72 h. The weight of swollen samples was determined after removal of the surface liquid with lint-free tissue paper. The water uptake capacity shown by crude xanthan gum was lower than modified xanthan gum. The water uptake capacity of chemically grafted xanthan gum was 110%, whereas for irradiated and crude xanthan gum was 96.3% and 68.22% respectively. Increased water uptake capacity of modified xanthan gum might be due to formation of a rigid network structure at higher concentration of crosslinking agents. Results are shown in table 4.

Viscosity measurement:

The viscosity of a fluid can be defined as the resistance generated during flow of liquid. In order to determine the rheological characteristics crude and modified xanthan gum viscosity measurement was performed. Viscosity results of crude xanthan gum, chemically grafted xanthan gum, and irradiated xanthan gum were found to be 1050 cP, 1680 cP and 1650 cP respectively. It is clear from the result that the viscosity of the crude gum was less as compared to grafted gum and irradiated xanthan gum, but there was no much variation in the viscosity of grafted gum and irradiated xanthan gum. The maximum viscosity was achieved for grafted gum and irradiated xanthan gum as compared to crude gum, which indicates modified gum is having maximum grafting efficiency. Higher grafting might be the fact that acrylamide side chains attach with the polymer backbone and form a branched network with xanthan gum which leads to increase the viscosity. In order to determine viscosity change, crude and modified xanthan gum samples were stored at room temperature and results showed that crude xanthan gum showed huge decrease in viscosity. There was slightly change in the viscosity of modified xanthan gum sample (table 4). From these results it was clear that modified xanthan gum preserves its viscosity for longer duration of time.

Table 4: Results of water uptake capacity and viscosity

Sample name	Water uptake (%)	Viscosity (cP)	
		Before storage	After storage
Crude xanthan	68.22	1050	760
Grafted xanthan	110	1680 cP	1675
Irradiated xanthan	96.30	1650 cP	1630

CONCLUSION

Modification of the nature of native gum is a novel approach, where native gum is treated with different chemicals or different wavelength radiations. In this present study xanthan gum was selected as model polymer and treated with acrylamide and microwave radiation. Characterization studies like FTIR, DSC, SEM and XRD results were evidence for the modification of crude xanthan gum. The grafted xanthan gums were biodegradable and having higher water uptake capacity, viscosity and grafting efficiency. In future, the study will continued with the formulation of controlled and sustain release dosage forms.

ACKNOWLEDGMENTS

Authors are thankful to RGUHS, Bangalore for providing fund to carryout this project.

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