Microwave Irradiated Copolymerization of Xanthan Gum with Acrylamide for Colonic Drug Delivery

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Xanthan gum (XG) is a polysaccharide produced by *Xanthomonas campestris*. The aim of the present study was to modify the xanthan by hydrolysis and grafting with acrylamide through microwave irradiation for different time intervals. Pure xanthan was partially hydrolyzed *via* enzymatic and chemical treatments followed by optional grafting. Proximate composition analysis, moisture content, and carbohydrate, protein, lipid, and fiber contents were determined. The morphological characteristics, structural composition, functional groups, and heat resistance of the crude, hydrolyzed, and grafted gum were evaluated using SEM, XRD, FTIR spectroscopy, and TGA. Morphological studies revealed that xanthan was broken down into smaller fragments as a result of hydrolysis and became somewhat smoother. Thermal analysis studies indicated a larger heat tolerance in the grafted xanthan relative to that of the native and hydrolyzed gums. Xanthan bound to a triamcinolone drug was evaluated in the context of controlled drug release. Controlled drug release correlated well with the exposure time to microwaves used to graft the gum.

Keywords: Xanthan gum; Acrylamide; Hydrolysis; Grafting; Triamcinolone

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INTRODUCTION

Natural gums are polysaccharides with noteworthy physical and chemical characteristics. Plants are the source of many natural gums. Gums give structural support to plants and act as energy reservoirs for them. These gums consist of simple repeating subunits of monosaccharides such as glucose, xylose, and fructose, which combine to form complex polysaccharides. Gums are being used commercially in some drugs as well as for laboratory research in the biochemistry and pharmacology disciplines because of their biodegradability and biosafety characteristics (Lachke 2004). Collection of these gums is expensive and requires special expertise. Their quality and prevalence can be affected by environmental changes. For these reasons, it may be advantageous to employ an alternative approach: obtain these polysaccharides from microbes and then modify them to increase their utility (Mundargi *et al.* 2007).

Xanthan is a high-molecular weight biopolymer produced by pure culture fermentation of carbohydrates with natural strains of *Xanthomonas campestris*. Xanthan

is available in powder form and has a milky color. It forms highly viscous, aggregated clusters in solution when dissolved in water, and the dissolved form is stabilized by hydrogen bonding (Srivastava *et al*. 2012). Xanthan gum was discovered in the mid-20th century by scientists of the U.S. Department of Agriculture developing microorganisms capable of producing commercially-relevant, water-soluble gums. This gum was first prepared on a commercial scale in 1960 (Pandey and Mishra 2011).

Xanthan, a polysaccharide, displays pseudoplasticity in aqueous solutions due to its helical structure. The predominant monosaccharide subunits present in this gum are D-mannose and D-glucose. It also contains pyruvic acid and D-glucuronic acid in the form of sodium or potassium salts. Xanthan in its dissolved form has a pH of around 7. Currently, several companies including Monsanto/Kelco and Rhodia are producing xanthan commercially. In recent years, China has also started producing xanthan. The annual production of xanthan was around 35,000 tons in 2001 (Sand *et al.* 2010). Xanthan is used abundantly in the food industry as a suspending and thickening agent for fruit pulps and chocolates. Some unique properties useful for modern food production, such as texture, viscosity, appearance, flavor, and water-control parameters, are improved by the addition of xanthan gum. When an aqueous solution of this gum is prepared, it exhibits less of a 'gummy' feel in the mouth compared to solutions of other gums. Xanthan and its derivatives have a wide range of applications in the chemical industry. Several researchers have studied the enzymatic hydrolysis of xanthan (Cheetham and Mashimba 1991; Adhikary and Singh 2004). Cellulase produced from fungal sources can only hydrolyze xanthan if it is in a largely disordered conformation. The hydrolysis of xanthan is assisted by ultrasonic radiation and enzymatic treatments.

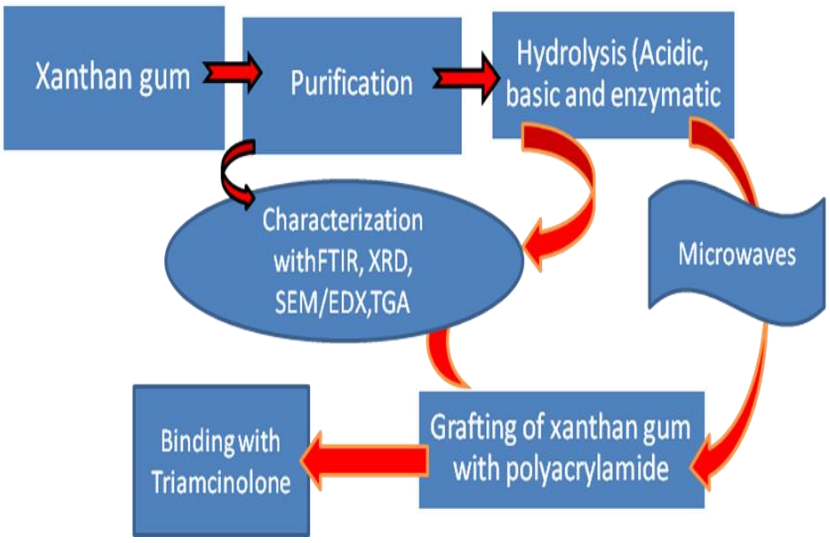

Fig. 1. Schematic diagram of grafting of xanthan gum

Although xanthan gum possesses a number of useful characteristics, it also has some drawbacks. For one, it is highly vulnerable to microbial attack. The vulnerability of xanthan to microbial attack can be minimized by grafting it with polymers such as acrylamide (Kumar *et al.* 2009). It is possible, in principle, to tune the rate of

biodegradation or chemical degradation to be suitable for selected applications. A number of efforts have been made to graft xanthan gum with different polymers. Grafting of xanthan gum is based on a free radical mechanism and different methods can be used to produce free radicals (Fig. 1). Some examples are the use of ions as free radical initiators or microwave radiation treatment. Most researchers working on the grafting of xanthan gum have grafted it with acrylamide and have studied its characteristics with respect to its drug release behavior as an active binder. Although the acrylamide monomer is a known strong neurotoxin, it is removed from the body in less than 24 h (Jampala *et al.* 2005). It is also known that the amount of unreacted acrylamide monomer can be minimized by suitable adjustment of the reaction conditions (Barvenik 1994).

Xanthan-based hydrogels are of prime importance mainly for biomedical application due to their biocompatible nature and similarities with biological systems. These can swell in biological fluids and water and can retain large amount of liquid in their swollen state. The present study is aimed at investigating the possibility of applying xanthan acrylamide-grafted gum to drug delivery for colon treatments. In order to do so, the xanthan gum was hydrolyzed and grafted with acrylamide using a microwave oven as a source of microwave radiation. In the process, the hydrolysis reaction of the gum and the structural characteristics of the modified product were investigated and measured.

EXPERIMENTAL

Materials

Xanthan gum was purchased from a local market in Faisalabad, Pakistan. The chemicals used were of analytical grade and were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich Chemical Co. (Buchs, Switzerland) unless otherwise stated. Acrylamide and triamcinolone (drug) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), while acetone and HCl were obtained from BDH (England). Methanol was bought from Merck (Germany).

Purification

Natural polysaccharides contain small amounts of other biomacromolecules such as proteins, fats, and fibrous materials as impurities. To remove these impurities, purification of the xanthan gum was carried out *via* various methods reported by Sandolo *et al.* (2007), Kwakye *et al.* (2010), and Dodi *et al.* (2011).

Proximate analysis of xanthan gum

The moisture contents, total carbohydrate, crude fat, crude protein, crude fiber, and ash contents of xanthan were determined following the methods of AOAC (2011).

Hydrolysis of xanthan gum

Acid and base hydrolyses of the purified gum were done according to the methods reported by Grossi *et al.* (2005) and Olga *et al.* (2008). To reduce the viscosity of the gum, enzymatic hydrolysis using cellulase was performed according to a method reported by Mudgil *et al.* (2012).

Grafting of xanthan

For the crosslinked grafting of the xanthan gum, the method of Kumar *et al.* (2009) was adopted. Xanthan gum (6 g) was carefully dissolved in 600 mL of distilled water using a magnetic stirrer. Acrylamide (30 g) was dissolved in 50 mL of distilled water. Both solutions were thoroughly mixed with a magnetic stirrer to obtain a homogenous mixture. This mixture was microwave-irradiated in a Dawlance microwave oven (DW-20M; Pakistan) for different time intervals (1 to 4 min). After microwave irradiation, the mixture was cooled and precipitated with an excess of acetone and then filtered. Precipitates of the grafted gum (XG-g-PAM) were dried in a hot air oven and ground to a fine powder with a mortar and pestle. This powder was further characterized by various analytical and structural techniques.

Swelling behavior of xanthan gum

The extent of swelling (swelling ratio and percentage) of the xanthan gum was calculated using the following equation,

$$\text{Swelling ratio} = W_{water} / W_{gel} \quad (1)$$

where W *water* is the sample weight after soaking and W *gel* is the sample weight after freeze-drying (Dodi *et al.* 2011). It can also be expressed as a percentage.

Methods

Fourier transform infrared (FTIR) analysis

The Fourier Transform Infrared (FTIR) spectroscopic analysis was performed to evaluate the different functional groups and the molecular structure of the native (crude), hydrolyzed, and grafted xanthan gum samples. The grafting onto the polymer backbone was confirmed by FTIR (Bruker, Impact 400 IR spectrophotometer; Germany). Analysis of the native, hydrolyzed, and grafted xanthan gum was recorded in a range from 400 to 4000 cm^{-1} (Mudgil *et al.* 2012).

X-ray diffractometry (XRD) analysis

X-ray diffraction analysis of the crude, hydrolyzed, and grafted gum samples in powder form was performed with a JEOL X-ray diffractometer (JDX 3532; Japan). The diffraction angle range of observation was 5 to 60°, with a scan step of 0.01 (Mudgil *et al.* 2012).

Scanning electron microscopy-electron dispersive X-ray spectroscopy (SEM-EDX) analysis

A JEOL SEM (JSM 5910; Japan) operating at an accelerating voltage of 10 kV was used to analyze the surface morphology, while EDX analysis (electron dispersive X-ray spectroscopy; Hitachi S-2380 N, Japan) yielded semi-quantitative amounts of the various elements present on the surfaces of the crude, hydrolyzed, and grafted xanthan gums (XG-g-PAM) (Sen *et al.* 2010).

Thermogravimetric analysis (TGA)

Thermogravimetric and differential thermal analysis (Shimadzu TGA/DTA-50, Japan) was used to evaluate the effect of temperature on the crude, hydrolyzed, and grafted samples of xanthan gum. Analyses were carried out across a temperature range of

30 to 1200 °C, with a uniform heating rate of 10 °C/min. The TGA studies were conducted in an inert nitrogen atmosphere (Singh *et al.* 2009).

High-performance liquid chromatography (HPLC) of hydrolyzed xanthan gum

The glucose and mannose contents of the hydrolyzed xanthan samples were determined *via* HPLC (Hitachi, Japan) using a Rezex RCM-Monosaccharide Ca^{+2} phenomenex column (Hitachi L 2130, Japan) with double-distilled (DD) water as the mobile phase at a temperature of 80 °C and a flow rate of 0.6 mL/min. A refractive index detector (Hitachi L 2400, Japan) was used for this analysis (Jahanbin *et al.* 2012).

Hemolytic activity

The toxicities of the samples were determined using the rapid hemolytic assay. A 3-mL sample of human blood from a volunteer following consent was collected, poured into a 15-mL screw-cap tube, and centrifuged for 5 min according to the method described by Powell *et al.* (2000). The supernatant was collected and the viscous platelets were washed three times with 5 mL of phosphate buffered saline (PBS) for the removal of platelets to obtain the purified erythrocytes. The washed cells were suspended in 20 mL of chilled, sterile PBS solution, and the cells were counted on a hemacytometer. The red blood cells (~1 $x10^8$) were suspended and maintained in ice cold PBS. Similarly, bovine blood was also processed to obtain the erythrocytes. Twenty-microliters of the gum samples were placed into aseptic 2.0-mL microfuge tubes. Each diluted blood cell aliquot (180 µL) was then placed into the 2-mL tube with the gum samples and carefully mixed three times with a wide mouth pipette tip. Tubes were agitated for 35 min in an incubator at 37 °C and then moved onto ice for 5 min before being centrifuged again for 5 min. One hundred microliters of the supernatant was collected, carefully placed into a sterile 1.5-mL microfuge tube, and diluted with 900 µL of chilled, sterile PBS. All tubes were maintained on ice after dilution. The absorbance of each sample was measured at 576 nm. The results were presented as percentage (%) hemolysis. Polyoxyethylene octyl phenyl ether (Triton-X-100) at a concentration of 0.1% was used as the positive control, and PBS as the negative control. All the samples were analyzed independently in triplicate for this activity (Shahid *et al.* 2013).

Preparation of particles

The polymer particles containing triamcinolone (TC) were prepared by complex ionotropic gelation under optimized encapsulation efficiency. Triamcinolone (5.0 mg/mL) was added to the xanthan polymer dispersion (1.00%) and agitated to obtain a uniform suspension of TC drug in the polymeric dispersion. The dispersion was mixed into the chitosan dispersion containing calcium chloride. The chitosan dispersion medium, with a pH 4.8, was prepared by adding 0.5% chitosan to 0.1 M acetic acid and stirring with a magnetic stirrer. Then, 1.5% of calcium chloride was added to the mixture.

In vitro study of drug release by using dissolution method

From particles, the *in vitro* drug release was analyzed on a Hanson SR8 dissolution station (Hanson Research; USA), using a basket apparatus. For the motivation of gastric or enteric media, 900 mL of HCl (0.1 M, pH 1.2) or phosphate buffer (0.5 M, pH 7.4) were used at 37 °C and with agitation speed of 50 rpm. About 40 mg of TC containing particles were placed in a basket, the HCl or phosphate buffer (2.5 mL) was withdrawn after appropriate time intervals, and drug release was assayed using a

spectrophotometer at 242 nm. The xanthan gum (XG) matrix was used as a reference, and each experiment was conducted in triplicate. After 2 h in gastric medium, the basket was removed and placed immediately in 900 mL of the following receptor medium and kept for another 4 h. The phosphate buffer (10 mM and pH 7.4) was used for evaluating release profile of TC (Shahid *et al.* 2013).

RESULTS AND DISCUSSION

Purification of Xanthan Gum

Purification and hydrolysis methods were used to remove any impurities or insoluble fractions present in the xanthan gum. The gum was purified using a Soxhlet extraction method with ethanol as a solvent (XG1) (Dodi *et al.* 2011), by magnetic and mechanical stirring using distilled water as a solvent (XG2) (Sandolo *et al.* 2007), and finally, by magnetic stirring using deionized water (XG3) (Kwakye *et al.* 2010) (Fig. 2).

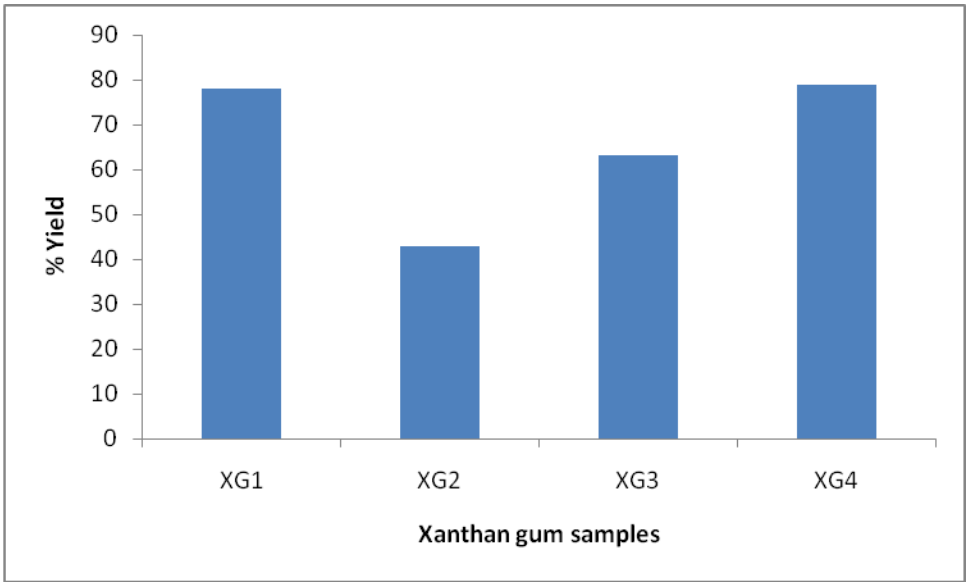

Fig. 2. Purification yield of xanthan gum by different purification procedures

The final purification method consisted of successive dissolution, stirring, precipitation, and filtration (XG4) (Kwakye *et al.* 2010). The purification yield depended on the method used: XG4 > XG1 > XG3 > XG2. The yield is the most vital economical feature of polysaccharide purification. The yield was highest in XG4 due to the long time period used to dissolve the crude sample, or possibly due to the recovery of almost all of the precipitated polysaccharide using ethanol (Fig. 2).

Proximate Analysis of Xanthan Gum

The proximate analysis of the crude gum shows that the carbohydrate content was 79.70%; this result indicates that the crude gum contained a substantial concentration of xanthan. However, it is susceptible to microbial attack due to the presence of 2.0% moisture. The ash content was found to be 12.0%, an indication of mineral content in the crude gum. The fats and proteins accounted for 5.4 and 0.9%, respectively, an indication of the nutritional value of the gum that make it suitable for food industry applications.

Hydrolysis of Xanthan Gum

The high viscosity of solutions of gum in their native form can limit their applications in some food products. To reduce the viscosity and extend its range of application, crude xanthan gum was modified by chemical and enzymatic methods. Partially hydrolyzed xanthan gum (PHXG) was produced to provide a dietary fiber source that can easily be added to food. The PHXG has a low molecular weight and viscosity, can easily be added to any diet, and is acceptable to consumers (Grossi *et al.* 2005). Its low viscosity, small amount of remaining glutaraldehyde, and thermal stability indicate that the xanthan hydrogel has potential as a biomaterial with satisfactory rheological properties (Cunha *et al.* 2007).

Grafting of Xanthan Gum with Acrylamide (XG-g-PAM)

The XG-g-PAM was synthesized *via* free radical formation onto the backbone of the xanthan gum through microwave irradiation (700 W) for different time intervals. Grafting with polyacrylamide is favored due to xanthan's high susceptibility to grafting reactions. Table 1 shows that the grafting rate, addition, and transformation increased with an increase in the exposure time to microwave irradiation up to 3 min. After this time, degradation of the polysaccharide backbone took place, resulting in homo-polymer formation. Grafting effectively occurred following microwave irradiation for 3 min (XG-g-PAM 3, 61%). Graft copolymerization was judged to be a suitable way to increase the utility and applications of this modified natural polymer.

Table 1. Grafting of Xanthan Gum with Acrylamide (XG-g-PAM) Irradiated with Microwaves at Different Time Intervals

Serial No.	Xanthan gum (g)	Microwave exposure time (min)	Microwave power (W)	Grafting (%)	Grafted polymer
1	2	1	700	14	XG-g-PAM 1
2	2	2	700	25	XG-g-PAM 2
3	2	3	700	61	XG-g-PAM 3
4	2	4	700	54	XG-g-PAM 4

Optimized grafting minimized the formation of homo-polymers and resulted in the proliferation of free radical sites on the backbone of the polymer. However, prolonged exposure to microwaves can disrupt the polysaccharide backbone and can result in homo-polymerization rather than graft copolymerization, as reported by Sen *et al.* (2010). When exposed to microwave radiation, free radical formation takes place due to the splitting of polar functional groups such as the OH bonds present on the backbone of the polysaccharides. Water molecules present in the polysaccharide structure are also microwave active but produce heat instead of free radicals due to the absorption of microwave irradiation. To avoid the formation of water molecules, the hydrophobicity or water incompatibility of the gum can be increased using suitable substitutions to the polysaccharide surface. Xanthan gum grafted with polyacrylamide had greater hydrophobicity and lower viscosity in aqueous solution than crude gum, in agreement with results reported by others in the field (Rana and Matsuura 2010).

Swelling Behavior of Xanthan Gum

Natural polysaccharides are hydrophilic and swell to form highly viscous

solutions or dispersions. Their successful application in nutraceutical products is dependent on their degree of hydration and swelling (Cunha *et al.* 2007), which is ultimately dependent on factors such as pH and temperature. The crude gum had a higher swelling ratio relative to the purified, hydrolyzed, and grafted xanthan gums (Table 2). Similar findings were reported by Nep and Conway (2010) and Dodi *et al.* (2011).

Table 2. Swelling Ratios of Xanthan Gum Samples

Sr. no.	Xanthan gum sample	Swelling ratio (g)
1	Crude	3.01
2	Purified	2.79
3	Acid hydrolyzed	2.51
4	Base hydrolyzed	2.57
5	XG-g-PAM 1	2.45
6	XG-g-PAM 2	2.28
7	XG-g-PAM 3	1.90
8	XG-g-PAM 4	2.13

Fourier Transform Infrared Spectroscopy

The FTIR spectra gave information regarding the vibrational frequencies of functional groups present in the polymer segments resulting from intermolecular interactions. The FTIR spectra of acidic-, basic-, and enzymatically-hydrolyzed and polyacrylamide-grafted xanthan gum were recorded to interpret the functional groups present within them and to investigate what structural changes occurred following the hydrolysis and grafting of the xanthan gum.

As shown in Table 3, the FTIR spectrum of the crude xanthan gum exhibits a prominent peak at 3505 cm^{-1}, which can be attributed to the region of hydrogen-bonded OH groups interacting with water molecules. The peaks at 2871 cm^{-1} and 2344 cm^{-1} are due to the C-H stretching vibrations of the $-CH_2$ groups in the xanthan gum.

Table 3. Possible Functional Groups Identified by FTIR Spectra in Crude, Acid Hydrolyzed, Base Hydrolyzed, Enzymatically Catalyzed, and Polyacrylamide Grafted Xanthan Gum

Xanthan gum									
Crude		Acidic hydrolyzed		Basic hydrolyzed		Enzymetically hydrolyzed		Polyacrylamide grafted	
Band intensity cm^{-1}	Functional group	Band intensity cm^{-1}	Functional group	Band intensity cm^{-1}	Functional group	Band intensity cm^{-1}	Functional group	Band intensity cm^{-1}	Functional group
3501	OH str vib.	3349	OH str vib.	3636	OH str. vib.	3501	OH str. vib.	3501	OH str vib.
2871	CH-str. vib. of CH2	3508	OH str vib.	3501	OH str vib.	3317	OH str. vib.	3397	NOH str. Vib. OH str. vib.
2344	CH-str. Vib. of CH2	3078	CH- str vib. Of CH2	2344	CH-str. Vib. of CH2	3206	OH str. vib.	2344	CH-str. Vib. of CH2
1695	CO str. Vib. of COOR	1683	CO str. vib.	1695	CO str. Vib. of COOR	1635	CO str. vib.	1693	CO str. vib.
1610	COO- str vib. of COOR			1610	COO- str vib. of COOR			1690	NOH ben. Vib.
1063	CO str. vib. Of COC			1063	CO str. vib. Of COC			1423	CN str. vib

Another peak at 1695 cm^{-1} can be ascribed to the C=O stretching vibrations of esters. The peak at 1610 cm^{-1} is attributed to COO^- stretching vibrations of esters (Nep

and Conway 2010). Absorption at 1063 cm^{-1} may be due to C-O stretching of the C-O-C linkage of glycosidic bonds. Similar results have been reported by others (Adhikary and Singh 2004; Nep and Conway 2010; Srivastava *et al.* 2012).

The FTIR spectrum of the acid-hydrolyzed xanthan gum shows peaks representing O-H stretching vibration at 3349 cm^{-1} and 3508 cm^{-1} and another peak at 3078 cm^{-1} due to C-H stretching vibrations of $-CH_2$ groups. The peak at 1683 cm^{-1} indicates the presence of C=O groups. There was no major transformation of functional groups as a result of acidic hydrolysis. In the spectrum of base-hydrolyzed xanthan, an additional peak at 3636 cm^{-1} was observed, which might be due to a non-bonded hydroxyl group of the base used for the hydrolysis. Enzymatically-hydrolyzed samples showed H-bonded O-H stretching vibrations at 3501 cm^{-1} and normal polymeric O-H stretching vibrations at 3317 cm^{-1} and 3206 cm^{-1}. The peak at 1635 cm^{-1} is due to the presence of C=O groups. Polyacrylamide-grafted xanthan exhibits some peaks in addition to those previously observed. Peaks near 1693 and 1690 cm^{-1} are attributed to the amide-I (C-O stretching) and amide-II (NOH bending) of the amide group of the polyacrylamide (PAM). The band at 1423 cm^{-1} is due to C-N stretching. The peak at 3397 cm^{-1} in the XG-g-PAM is attributed to the overlapping of the NOH stretching band of the amide group and the OH stretching band. These peaks are evidence that crosslinked grafting took place successfully, as these results are similar to those reported by other scientists with reference to the same mechanism (Adhikary and Singh 2004; Nep and Conway 2010). The same mechanism of grafting has also been reported in our previous work (Shahid *et al.* 2013).

X-Ray Diffraction Analysis of Crude, Hydrolyzed, and Grafted Xanthan Gum

X-ray diffraction analysis was performed to determine the crystallinity of the crude, hydrolyzed, and grafted xanthan gums. The diffractograms reported in Fig. 3 show that microwave irradiation-initiated grafting increased the crystallinity of the xanthan gum.

The crude xanthan gum was found to be largely amorphous and had only two crystalline peaks in the 2θ range of 32.0513 to 35.210°, with relative intensities of 58 and 96%, respectively. The grafted copolymer (XG-g-PAM 3) displayed six visible, distinguishable diffraction peaks at 2θ values of 12.132, 19.0327, 23.539, 28.457, 42.132, and 49.028° compared to the two peaks of the crude xanthan gum. This verifies that the maximum grafting was achieved with acrylamide to obtain polyacrylamide grafted xanthan gum product. The peaks had relative intensities of 100.910, 40.189, 35.639, 76.492, 42.555, and 64.570, respectively. An increase in crystallinity following graft copolymerization of modified gum was also detected by Singh *et al.* (2009) and Sharma and Lalita (2011).

In contrast to our results, Mishra and Kumar (2011) recorded no distinguishable crystalline peaks in the modified gum. The X-ray diffractogram of the acid-hydrolyzed xanthan gum did not show any definitive crystallinity peaks, indicating that the hydrolysis resulted in a loss of crystallinity and that the hydrolyzed gum became amorphous.

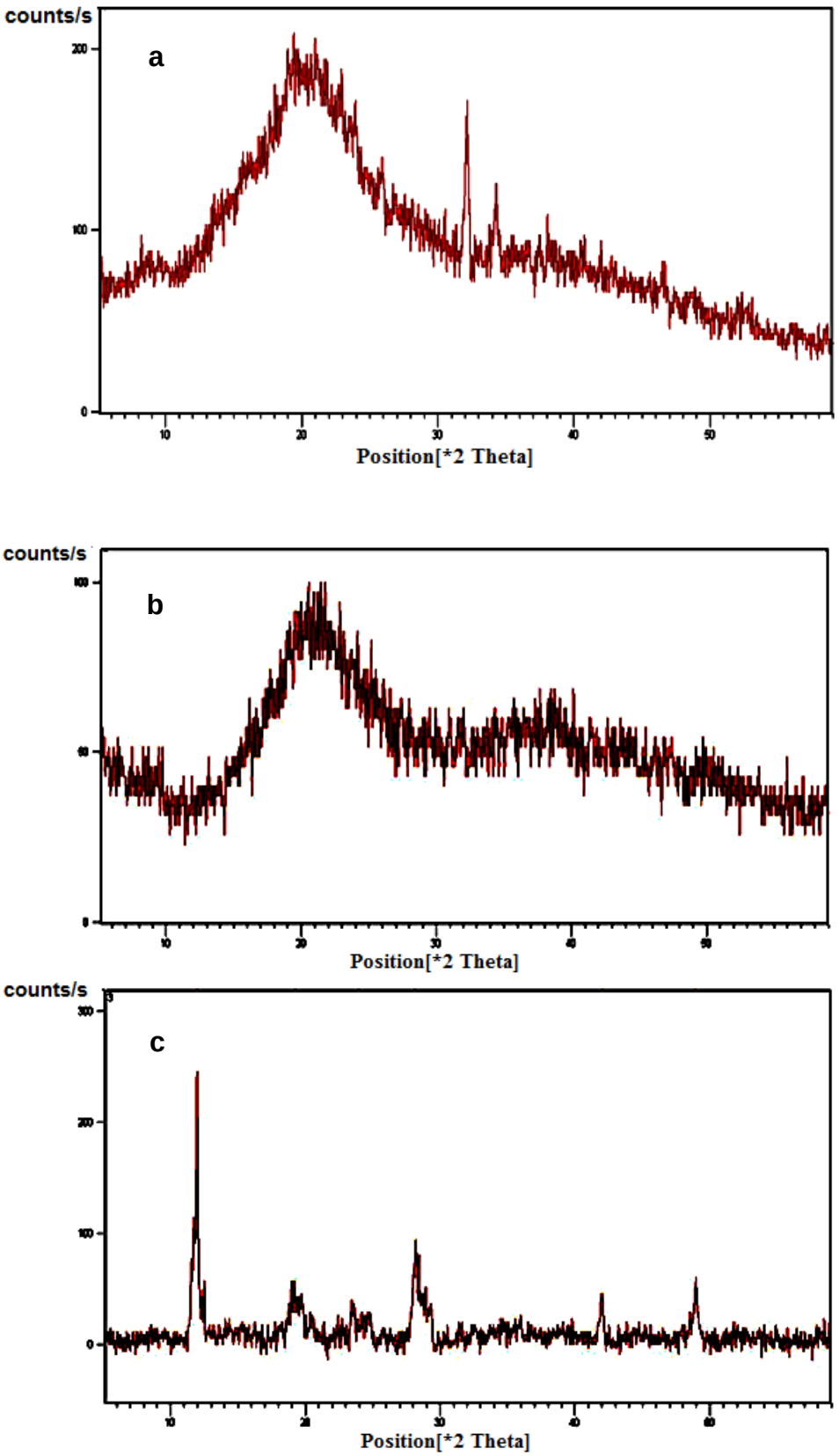

Fig. 3. X ray diffraction pattern of crude (a), acid hydrolyzed (b), and grafted (c) xanthan gum

Scanning Electron Microscopy Analysis

Scanning electron microscopy was performed to investigate the detailed morphology of the surfaces of the native gum, its hydrolyzed products, and the modified (grafted) gum (Fig. 4).

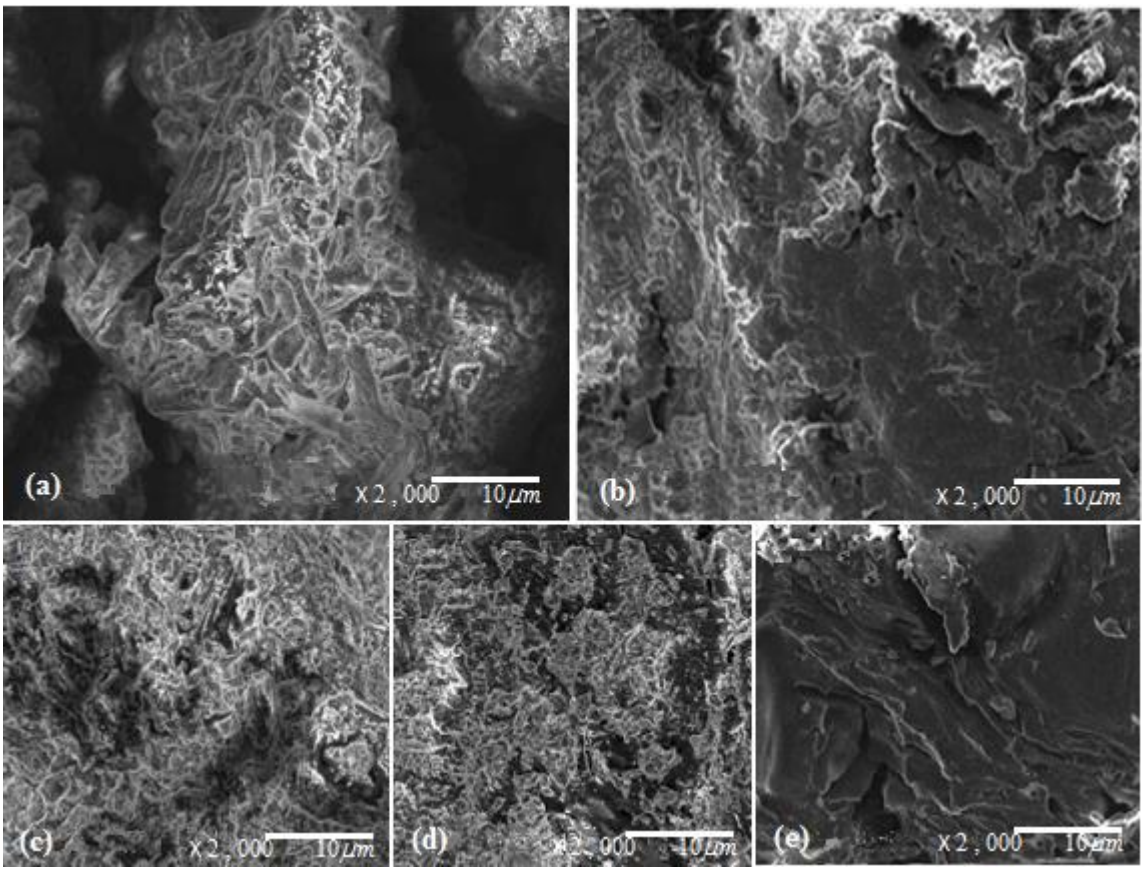

Fig. 4. Scanning electron micrographs of (a) crude, (b) acid hydrolyzed, (c) base hydrolyzed, (d) enzymatically hydrolyzed, and (e) grafted xanthan gum

The xanthan gum had a fibrous surface morphology. Fibers seemed to be present in associated forms. A fibrous surface morphology was also detected in the acidic, basic, and enzymatic hydrolyzed xanthan gums. However, the thickness of these fibers decreased and they seemed more homogenous.

A decrease in the thickness of the fibers could be because the xanthan gum was broken down into smaller units as a result of hydrolysis. The grafted sample of xanthan gum (Fig. 4e) showed a somewhat smoother morphology pattern and enhanced fibrous structure.

Energy dispersive X-ray spectroscopy (EDX) was carried out to detect the elements present on the surfaces of particular areas of the xanthan gums (Fig. 5). From the EDX analyses, it is clear that the xanthan gum sample consisted largely of carbon (47.1%) and oxygen (48.0%) with smaller concentrations of sodium (2.4%), calcium (1.9%), and sulphur (0.6%).

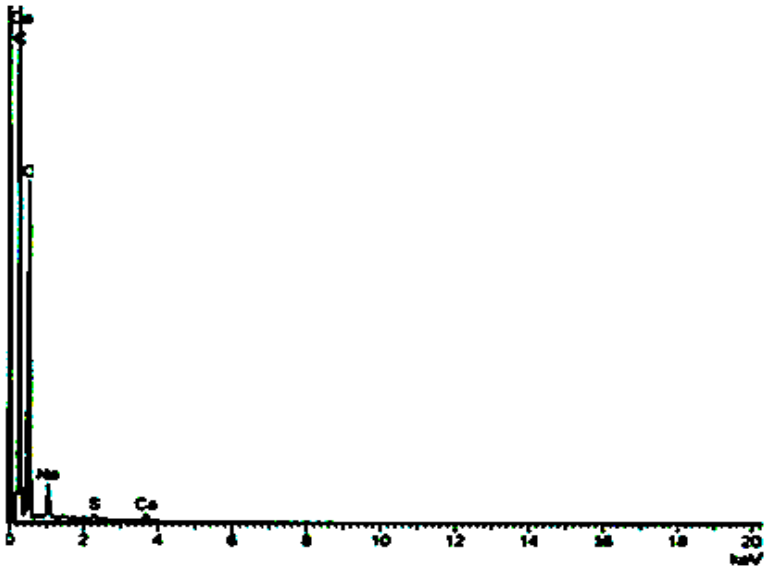

Fig. 5. Electron dispersive x-ray pattern of elements present on the exposed surface of crude xanthan gum

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was performed to illustrate the changes in the mass gum samples with respect to temperature or time (Fig. 6). Xanthan gum exhibited a complex degradation pattern. About 13% mass loss occurred below 160 °C. This loss of mass might be due to desorption of water (Fig. 6a). After the initial mass loss, the mass remained constant for some time before further decomposition began at about 200 °C. Almost half of the mass loss occurred by about 330 °C, above which the rate of mass loss declined. Approximately 85% of the total mass loss occurred before reaching a temperature of 700 °C.

In contrast to the present results, Banerjee *et al.* (2006) reported a somewhat different pattern of degradation. Despite the different degradation pattern, the rate of mass loss was quite similar to those of earlier observations (Adhikary and Singh 2004; Srivastava *et al.* 2012). For the acid-hydrolyzed xanthan gum (Fig. 6b), it was observed that decomposition began almost immediately. This may be because the gum was already dehydrated during the acid hydrolysis process.

The rate of mass loss was comparatively fast. About 90% of the mass loss occurred below 550 °C. From 800 °C onwards, there was very little mass loss. Thus, acid-hydrolyzed gum is less stable, perhaps as a result of fragmentation during acid hydrolysis. In the base-hydrolyzed xanthan gum, degradation began at 250 °C (Fig. 6c). In this case the rate of mass loss was lower than that in all previous samples. About 70% of the mass loss occurred below 600 °C. In the enzyme-hydrolyzed xanthan gum, degradation started at 200 °C (Fig. 6d). The initial mass loss was 14% at 150 °C and can be attributed to desorption of water. The pattern of mass loss was very similar to that of the native xanthan gum.

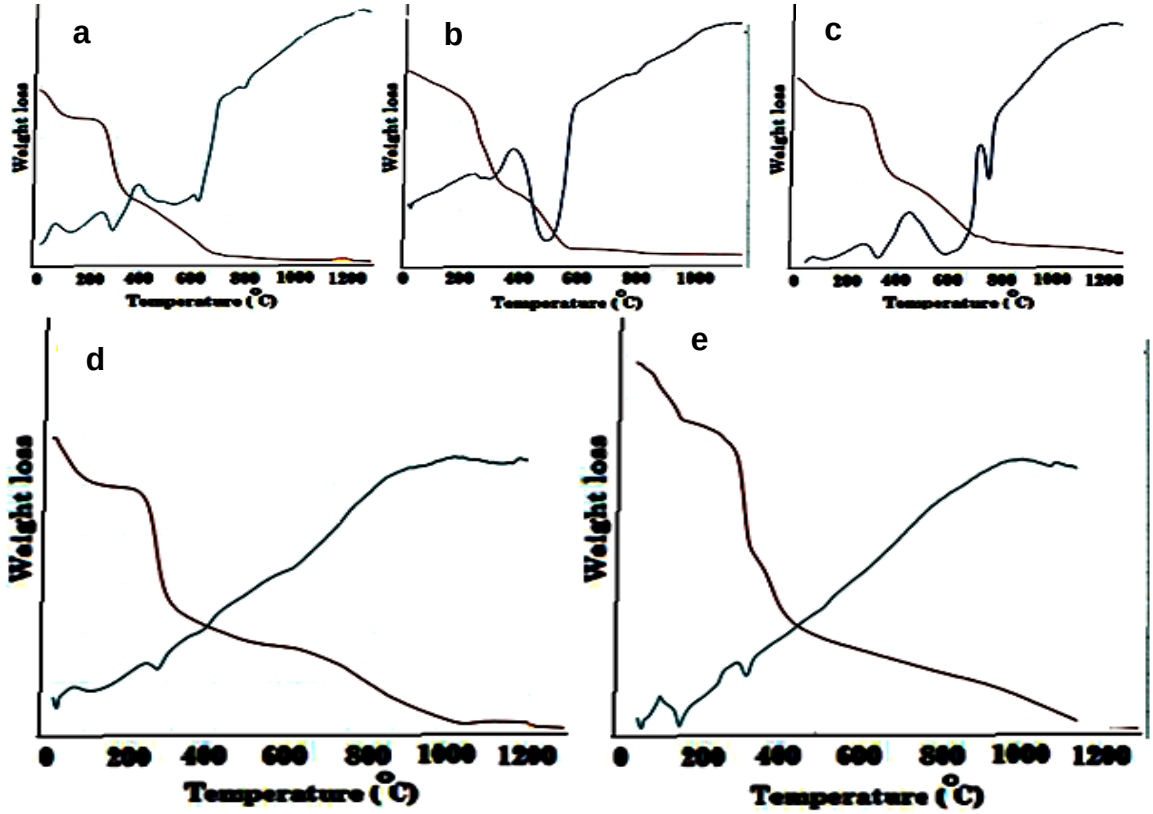

Fig. 6. TGA curves of (a) crude, (b) acid hydrolyzed, (c) base hydrolyzed, (d) enzymatically hydrolyzed, and (e) acryl amide grafted xanthan gum

The TGA output for the XG-g-PAM (Fig. 6e) shows that initially (at 170 °C) almost 16% mass loss occurred due to desorption of water before true degradation began at 250 °C. About 75% of mass loss occurred below 450 °C. Thereafter, the rate of mass loss decreased. The grafted gum exhibited greater thermal stability than the native gum in terms of the degradation starting point. These results are similar to those reported by Srivastava *et al.* (2012).

High-Performance Liquid Chromatography (HPLC) Analysis in Acid Hydrolyzed Xanthan Gum

The HPLC analysis of the xanthan gum (Table 4) was carried out to determine the glucose and mannose (monosaccharide subunits of xanthan gum) contents. The glucose content was found to be 1.22%, whereas that of mannose was 1.25%. They were present at a 1:1 ratio, which confirms earlier results (Mishra and Kumar 2011). However, these results are in contrast with those of some other reports (Adhockery and Singh 2004; 2011; Banerjee *et al.* 2006; Nep and Conway 2010) in which it was noted that the glucose:mannose ratio exceeded 1:1 in acid-hydrolyzed xanthan gum.

Table 4. Compounds Detected by HPLC Analysis of Acid Hydrolyzed Xanthan Gum

Compounds	Retention time	Area (%)	% age
Glucose	11.203	13.5	1.22
Mannose	12.947	18.3	1.25

Hemolytic Activity

The crude, hydrolyzed, and grafted xanthan gum samples were screened using a commercial rapid assay against human and bovine erythrocytes. The results are summarized in Table 3. No toxicity was observed in the crude and acid hydrolyzed samples of the gum. Very low hemolytic activity was recorded in the base-hydrolyzed, enzymatically-hydrolyzed, and grafted samples (Table 5). The mechanical stability of the membrane of red blood cells (RBCs) is a good indicator to evaluate *in vitro* cytotoxic effects of the gums. Treating cells with a toxic gum can cause different problems to human beings as the cells may undergo a loss of membrane integrity and die rapidly as a result of cell lysis. The toxicity of xanthan gums has not been detected previously by any other author (Shahid *et al*. 2013).

Table 5. Hemolytic Activity (%) of Crude, Purified, and Grafted Xanthan Gum Samples Against Human and Bovine Erythrocytes

Samples	Human erythrocytes	Bovine erythrocytes
Crude xanthan gum	ND	1.01
Acid hydrolyzed	ND	ND
Base hydrolyzed	1.03 ± 0.31	1.00 ± 0.42
Enzyme hydrolyzed	0.51 ± 0.09	ND
XG-g-PAM 1	1.0 ± 0.42	1.11 ± 0.19
XG-g-PAM 2	1.03 ± 0.28	1.5 ± 0.51
XG-g-PAM 3	1.7 ± 0.41	2.41 ± 0.5
XG-g-PAM 4	2.01 ± 0.72	1.51 ± 0.47
Phosphate buffer saline	ND	ND
Triton-X-100	99.71 ± 0.65	100 ± 0.53

Values (mean ± SD) are average of three samples, analyzed individually in triplicate (n = 1 x 3 x 3)
ND: not detected

In Vitro Drug Release Studies

Grafted xanthan gum was bound to the drug triamcinolone, and the drug release profile was compared in gastroenteric and colonic systems (Fig. 7). No release of any of the formulated drugs into the gastroenteric system was observed during the first 40 min of the assay. During the first hour, the drug release from XG-g-PAM 1 was 6% compared to 5% from XG-g-PAM 2 and XG-g-PAM 4.

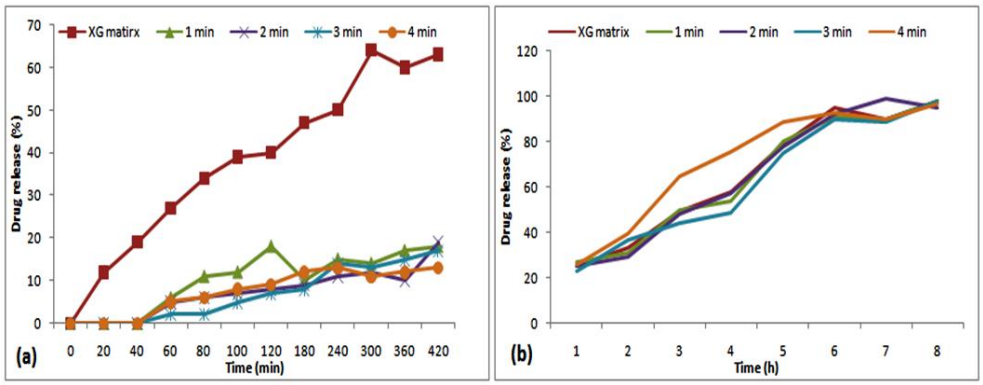

Fig. 7. (a) *In vitro* drug (triamcinolone) binding in the gastro-enteric system and (b) *in vitro* drug (triamcinolone) binding in the colonic system

A low drug release (2%) and 27% XG matrix was observed from the XG-g-PAM 3, which indicated that it yielded the strongest control over drug release. It is notable that even one minute of grafting time, corresponding to a grafting level of 14%, was sufficient to delay the onset of release by 60 min in an environment representing a stomach. This is a highly promising result, suggesting that it is possible to suppress the release of drugs as desired while minimizing the amount of monomeric acrylamide units introduced to the system.

In the colonic system, the measured drug releases were 27% from XG-g-PAM 1, 25% from XG-g-PAM 2, 23% from XG-g-PAM 3, and 26% from XG-g-PAM 4. Drug release was higher in the colonic medium than in the gastro-enteric medium. Drug release from XG-g-PAM 3 was 14 and 49% during 4 h of assay in the gastro-enteric and colonic systems, respectively, whereas from XG matrix the corresponding values were 50 and 58%. The higher release under the alkaline conditions of the colon is consistent with the dissociation of the carboxyl groups of the hydrolyzed xanthan, leading to higher swelling and solubilization.

The delay of drug release in the acidic environment might be due to the binding of the drug with XG-g-PAM 3, inhibiting swelling of the formulated drug (Kumar *et al.* 2009). It is clear from these drug release profiles that the gum with the highest grafting percentage (XG-g-PAM 3) delayed drug release the most. With increased grafting, a more complex structure of the gum was formed, resulting in more effective intermingling of chains. This complex structure did not allow the rapid release of the formulated drug and resulted in slower release of the enclosed drug. Acrylamide itself is a neurotoxin used in the grafting of xanthan gum (Caulfield *et al.* 2002). Polyacrylamide degrades slowly (10% in 28 days), but fortunately, the transit time for an oral formulation to the gastrointestinal tract is just 24 h. Therefore, the compounds investigated in the present study are not expected to be harmful and will be excreted from the body normally along with other waste materials. In future studies, modified gum can be bound to other drugs and studied with respect to its drug-retarding ability compared to that of native gum. Moreover, xanthan can also be grafted with other polymers such as bisacrylamide using microwave irradiation in the future. The encapsulation of grafted gum with nanoparticles is a potential future topic of research

CONCLUSIONS

1. A relatively short grafting time (1 min), corresponding to a 14% grafting level, was sufficient to cause a 60 min delay in drug release in a gastric pH environment. However, drug release was more pronounced in the basic medium, simulating the colonic system.
2. The binding of grafted gum with drug particles reduced their swelling in media similar to the gastro-enteric and colonic systems. This modification suggests gum could improve orally administered drug delivery.
3. Binding of grafted xanthan gum to polyacrylamide resulted in greater control of the drug release in both media. An acidic medium delayed the release of the drug. Hydrolyzed and grafted gum exhibited no cytotoxicity, thus indicating it is safe for human use.

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