Synthesis of novel biodegradable antibacterial grafted xanthan gum

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A B S T R A C T

Xanthan gum (XG) is natural polysaccharides used in food industries as stabilizers and thickener agents. The problem is that some food products are found to be contaminated by pathogenic bacteria such as Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) that reduce their shelf life. This research aims to synthesize biodegradable antibacterial XG-grafted-poly(N-vinyl imidazole) PVI and the effect of reaction parameters were studied on grafting yield (G), grafting efficiency (GE), total conversion (TC) and homopolymer (H) %. XG-g-PVI was characterized via various analysis tools. Thermal analysis showed that grafted XG was more thermally stable than unmodified XG and their stability increased with increasing PVI%. XG-g-PVI was acting as antibacterial agent against (E. coli and S. aureus) bacteria that cause food borne diseases. Their activity increases with increasing grafting yield%. Surface morphology showed change from irregular lobules shape in XG to smooth surface in its graft with PVI.

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1. Introduction

In past decades, researchers were interested in natural biopolymer polysaccharides due to their availability, biodegradability, low cost and non-toxicity. Biopolymers are used in different fields like food and cosmetic industries (Bhardwaj, Kanwar, Lal, & Gupta, 2000; Badwaik, Sakure, Alexander, Dhongade, & Tripathi, 2016; Chen, Jo, & Park, 1995). Food production usually contains natural polysaccharides such as xanthan gum (XG) (Talukdar & Kinget, 1995; Xiong, Li, Xie, Xue, & Sun, 2014).

XG is one of the best antioxidant biopolymers that are used today in food industry like salad dressings, frozen foods, dairy products as stabilizer and thickener agent because of its biodegradability, biocompatibility and non-toxicity. XG is anionic biopolymer that is extracted from Xanthomonas campestris, so it is known as microbial polysaccharide (Fig. 1). Its structure consists of backbone of β-(1→4)-d-glucopyranose glucan and side chains of β-(3→1)-α-linked d-mannopyranose-(2→1)-β-d-glucuronic acid-(4→1)-β-d-mannopyranose on alternating residues and its monomer has molecular formula C35H60O29 (molecular weight (MW) ≈ 933 g mol−1) (Bilanovic, Starovetsky, & Armon, 2016; Garcia-Ochoa, Santos, Casas, & Gomez, 2000 Pandey & Ramontja, 2016; Talukdar & Kinget, 1995; Xiong et al., 2014).

However, food products are exposed to contamination by pathogenic bacteria such as Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus). Bacterial contamination reduces the shelf life of food products and increases the risk of food borne diseases (FBD) that it is considered global problem threatening the human health (Quintavalla & Vicini, 2002; Gurbuz, Saham, Kara, & Osman, 2009). Imidazole ring containing compounds showed good antibacterial activity due to its cationic nature besides they are biodegradable and thermally stable. Imidazole ring is main component of most biomolecules such as; histidine as amino acid, hemoglobin as protein and histamine as hormone. Synthetic polymers with imidazole ring, especially poly (N-vinyl imidazole), PVI, can be considered one of the most important antibacterial polymers nowadays (Anderson & Long, 2010; El-Hamshary et al., 2015; Fodor, Bozi Jn Blazsoi, & Ivan, 2012). Thus, we expect that grafted XG with imidazole ring containing monomer like; PVI will control pathogenic bacteria (E. coli and S. aureus) growth and improve XG thermal stability of products that leading to increase the shelf life of food products.

In the present work, we report on synthesis and characterization of biodegradable grafted xanthan gum (XG) with poly(N-vinyl imidazole) (PVI) via different analysis techniques such as FTIR, SEM, XRD and elemental analysis. Thermal stability of samples was examined using TGA. Also, various grafting parameters were studied, in addition to studying their antibacterial activity against two pathogenic bacteria (E. coli and S. aureus).
2. Materials & experimental methods

2.1. Materials

Xanthan Gum (XG) was purchased from Alpha-Chehika, India. N-Vinylimidazole, potassium persulfate (K$_2$S$_2$O$_8$), absolute ethanol and acetone were purchased from Sigma-Aldrich, Germany.

2.2. Experimental methods

2.2.1. Synthesis of XG-g-PVI

XG-g-PVI copolymer was prepared by free radical polymerization reaction under nitrogen atmosphere using PPS (K$_2$S$_2$O$_8$) as initiator. Briefly, 0.1 g of XG (W$_0$) was dissolved in 25 mL of distilled water under constant stirring. Different amount of PPS solution (15–55 × 10$^{-3}$ mol L$^{-1}$) were added dropwise to XG solution then different amount of vinyl imidazole (0.25–1.5 mol L$^{-1}$) were added to above mixture dropwise under stream bubbling of nitrogen gas at different temperatures (30–70 $^\circ$C). The grafting reaction was carried out during time range (1–3 h). The resultant graft was cooled to ambient temperature then was poured onto cold acetone under vigorous stirring for precipitation. The precipitated product was separated and left to dry in an air oven at 50 $^\circ$C till constant weight (W$_2$). The grafted product was extracted from hot absolute ethanol for 8 h using soxhlet apparatus to get rid of homopolymer. After extraction, purified copolymer products were dried in an air oven at 50 $^\circ$C to constant weight (W$_1$). The percentage of grafting yield (%G), grafting efficiency (%GE), total conversion (%TC) and homopolymer percentage (%H) were calculated according to the following Eqs (1)–(4) (Badwaik et al., 2016; Caner, Yilmaz, & Yilmaz, 2007; Pandey & Mishra, 2011).

\[
%G = \frac{[W_1-W_0]}{W_0} \times 100
\]

(1)

\[
%GE = \frac{[W_1-W_0]/[W_2-W_0]}{100}
\]

(2)

\[
%TC = \frac{[W_2-W_0]}{W_3} \times 100
\]

(3)

\[
%H = 100 - % GE
\]

(4)

Where, W$_0$ is initial weight of XG, while, W$_1$ and W$_2$ are weight of grafted matrix after and before purification, respectively; while, W$_3$ is the weight of monomer charged.

2.2.2. Antibacterial activity

Antibacterial activity of investigated samples (XG and XG-g-PVI with different% G) was evaluated against two pathogenic bacteria, Gram-positive bacteria as *S. aureus* (ATCC 12600) and Gram-negative bacteria as *E. coli* (ATCC 11775) using nutrient agar medium in presence of water as solvent control. Antibacterial test was carried out using the agar well diffusion method. Diameter of the well was 6 mm and the concentration of the investigated sample was 5 mg mL$^{-1}$. The plates were incubated at 37 $^\circ$C for 24 h. After incubation, antibacterial activity was estimated by measuring the inhibition zones diameter against the tested organisms. Inhibition diameter zones of antibacterial activity were expressed in millimeters (mm).

The experiment was done in triplicates; the average inhibition zone diameter was calculated and tabulated as mean plus standard deviation ($\pm$SD).

3. Instrumentation

Fourier Transform Infrared (FTIR) spectra of XG and XG-g-PVI copolymer were done using Jasco FTIR 4100 spectrometer (Japan) in the wavenumber range of 4000–600 cm$^{-1}$. Proton Nuclear Magnetic Resonance ($^1$H NMR) spectra of XG and grafted XG were determined by Varian Mercury (VX-300) NMR Spectrometer.$^1$H NMR spectra were run at 300 MHz in D$_2$O as solvent for XG and its copolymer. The powder X–Ray Diffraction (XRD) patterns of XG and copolymers were obtained using an x-ray powder diffractometer (a Philips Xpert MPD Pro) with Ni-filtered CuK$\alpha$ radiation of the wavelength ($\lambda$) of 0.154 nm at an accelerating voltage/current of 50 kV/40 mA and the relative intensity was recorded in the scattering range 2θ from 3 to 60° at a scan speed of 1 step s$^{-1}$. Thermal stability of XG and its grafted copolymers were determined using thermogravimetric analyzer (TGA) (TGA-50H Shimadzu). The measurements were done over temperature range of 25–700 $^\circ$C and the reference material was alumina with a dynamic heating rate 10 $^\circ$Cmin$^{-1}$ under N$_2$ atmosphere. All experiments were taken 4–5 mg of tested samples. The surface morphology of XG and grafted copolymers was examined by a field emission scanning electron microscope, FE-SEM, (Quanta 250 FEG) with a magnification of 1000 and accelerating voltage was 30 kV. Tested samples were prepared by placing a small part of them on a carbon tape on a stub, which they were coated with a thin layer of gold. Elemental analysis of XG and grafted copolymers was done using Elementar Vario ELIII Analyzer.

4. Results and discussion

4.1. Synthesis of the XG-g-PVI

The optimum reaction conditions were determined with varying one of the reaction parameters (PPS concentration, monomer concentration, reaction temperature and reaction time), while the others were kept constant under continuous stirring and in presence of N$_2$ gas supply — Fig. 2.

4.1.1. Effect of initiator concentration

Fig. 2a illustrates that KPS concentration affected on grafting parameters (%G, %GE, %TC and %H) with varying its concentration from 15 to 55 × 10$^{-3}$ mol L$^{-1}$, while the other reaction parameters were kept constant being monomer concentration (0.5 mol L$^{-1}$), temperature (50 $^\circ$C) and time (2 h). The results exhibited that all grafting parameters were increased with increasing the KPS concentration except %H. They reached a maximum value of around (%G 239%), (%GE 73%) and (%TC 63%) at 45 × 10$^{-3}$ mol L$^{-1}$ of KPS.

The grafting parameters increased with increasing initiator concentration because of increasing the active center (alkoxy XG radical) along XG backbone and followed by grafting of vinyl imidazole monomer onto the backbone (Scheme 1). At higher initiator concentration (above 45 × 10$^{-3}$ mol L$^{-1}$), % H was increased because initiator radical reacted with monomer and this reaction was more dominant than formation alkoxy XG radical (Scheme 1). This led to formation of PVI as homopolymer during the grafting process (Scheme 1), so, other grafting parameters were decreased. These results are in good accordance with those reported in literature (Badwaik et al., 2016).

Fig. 1. Chemical structure of Xanthan gum.
4.1.2. Effect of vinyl imidazole concentration

The effect of vinyl imidazole monomer concentration [M] on grafting parameters was studied by changing it from 0.25 to 1.5 mol L\(^{-1}\) while keeping the other reaction parameters constant (initiator concentration \(45 \times 10^{-3}\) mol L\(^{-1}\), temperature 50 °C) and time (2 h). The results are illustrated in Fig. 2b and they showed that all grafting parameters (except %H) were found to increase on increasing monomer concentration from 0.25 to 1.0 mol L\(^{-1}\) to a maximum values of (%G 444%), (%GE 81%) and (%TC 68%) at 1.0 mol L\(^{-1}\). According to the sequence of addition of the different reagents (see experimental Section 2.2.1), availability of large amount of monomer during chain propagation step in grafting copolymerization mechanism (Scheme 1) that led to increase %G. However, on further increasing the monomer concentration (above 1.0 mol L\(^{-1}\)) the graft yield starts to decrease, whereas the %H increase. This is probably due to the increase in the viscosity of the medium, which oppose the diffusion of the monomer towards the xanthan matrix, and consequently the residual monomers are consumed with formation of homopolymer. These observations were similar to other results in literatures (Kumar, Srivastava, & Behari, 2009; Pandey & Mishra, 2011).

4.1.3. Effect of temperature

The effect of reaction temperature on grafting parameters was studied through temperature range 30–70 °C, while, other reaction parameters were kept constant being [KPS] = \(45 \times 10^{-3}\) mol L\(^{-1}\), [M] = 1.0 mol L\(^{-1}\) and reaction time = 2 h and the results are shown in Fig. 2c. The results illustrated an increase in grafting parameters with the increase in the reaction temperature from 30 °C to 50 °C to reach the maximum grafting at 50 °C because rise of temperature led to increase the rate of decomposition of potassium persulfate to potassium sulfate radical (KSO\(_4\) radical) in initiation step (Scheme 1) that led to form more active sites (primary free radicals) on XG backbone and formed high %G when vinyl imidazole was added to XG/initiator solution. However, high temperature (above 50 °C), the grafting parameters decreased because K\(_2\)S\(_2\)O\(_8\) decomposed to O\(_2\) and H\(_2\)O as reported by literature (Kumar et al., 2009) that reacted with primary free radicals (alkoxy XG radicals) and increased rate of termination step (before the monomer addition) in Scheme 1. Consequently the concentration of alkoxy XG radicals decreased that led to decrease in grafting parameters which increased %H. These results were similar to the results in literatures (Lv et al., 2009; Trivedi, Kalia, Patel, & Trivedi, 2005).

4.1.4. Effect of reaction time

The reaction time affected grafting parameters and it was studied in time period range (1–3 h) keeping all the other reaction parameters constant (temperature was 50 °C, [KPS] was \(45 \times 10^{-3}\) and [M] was 1.0 mol L\(^{-1}\)). Results are shown in Fig. 2d clearly revealed an increase in %G, %GE and %TC with the increase in the reaction time up to 2 h, while %H decreased.
Due to increasing the active sites number on XG backbone, increase the reaction time (more than 2 h) had led to a decrease in grafting parameters (%G, %GE and%TC) because of reduction of initiator and monomer concentration which led to decrease the active sites on XG chains, these observations were similar to results in literature (Badwaik et al., 2016; Kumar et al., 2009).

4.2. Characterization of the XG-g-PVI composites

4.2.1. FTIR spectroscopy

FTIR spectra of XG and XG-g-PVI (%G 444%) are illustrated in Fig. 3. XG showed a broad band at 3431 cm⁻¹ corresponding to stretching vibration of hydroxyl (-OH) groups, while, another one appeared at 2922 cm⁻¹ corresponded to stretching band of aliphatic hydrocarbon (C−H) groups. Furthermore, absorption band at 1639 cm⁻¹ assigned for stretching carbonyl (C=O) groups of pyruvate and absorbation band at 1422 cm⁻¹ related to stretching symmetric carbonylate (−COO⁻) groups of gluconic acids. Also, a broad band at 1065 cm⁻¹ assigned for stretching vibration of ether (C−O−C) bond (Maitly & Sa, 2014; Pandey & Ramontja, 2016; Pandey & Mishra, 2012). While, FTIR spectrum of grafted XG with PVI exhibited band at 3120 cm⁻¹ due to stretching vibration of SP² hydrocarbon (C≡H) groups in imidazole ring. Also, a band appeared at 1582 cm⁻¹assigned for stretching ring olefin (C≡C) groups. In addition to, C≡N(ring) and C≡C stretching vibration bands appeared at 1496 cm⁻¹, band at 1422 cm⁻¹ referred to in-plane bending aliphatic (C−H) groups and two band at 1239 cm⁻¹ and 620 cm⁻¹ corresponded to stretching vibration of C−N bond of imidazole ring. Furthermore, sharp band appeared at 1113 cm⁻¹ assigned for in-plane bending vibration of C−H bond inside imidazole ring. Two bands for out-of-plane bending of C−H bond in imidazole ring appeared at 850 cm⁻¹ and 756 cm⁻¹ (Fodor et al., 2012; Goswami & Dutta, 2011; Pekel, Rzaev, & Güven, 2004; Pekel & Güven, 2008).

4.2.2. ¹H NMR spectroscopy

The structure of XG and XG-g-PVI (%G 444%) was proved using ¹H NMR in Fig. 4. ¹H NMR spectra of XG − Fig 4a − showed singlet signal at δ = 1.3 ppm corresponded to protons of methyl (−CH₃) groups and pyruvate methylene (−CH₂) groups in the side chains of XG (assigned by No. 1 in the chart) and methyl protons of acetylated groups (CH₃COO−) in XG side chains (assigned by No. 2 in the chart). In addition to a multiplet signal at δ = 3.0–3.4 ppm referred to protons of alcoholic methine (−CH−OH) and methylene groups (−CH₂−OH) (assigned by No. 3, 4 and 5 in the chart respectively). Also a sharp signal appeared at δ = 4.9 ppm related to anomeric protons which overlapped with the signal of D₂O solvent (Maitly & Sa, 2014; Mendes et al., 2011).

While ¹H NMR spectra of XG-g-PVI − Fig 4b− showed characteristic signals of PVI and XG structures. It illustrated characteristic signals of PVI, multiplet signals at δ = 6.9–7.6 ppm assigned to protons of imidazole ring (assigned by c, d and e in the chart), also signals of methine protons appeared at δ = 3.7–3.9 ppm as multiplet signals (assigned by b in the chart). A broad doublet signal of the backbone methylene protons appeared at δ = 2.2–2.5 ppm (assigned by a in the chart) (Pekel et al., 2004). Furthermore, ¹H NMR spectra of XG-g-PVI showed characteristic signals of XG such as signal of methyl protons and pyruvate methylene protons at δ = 1.3 ppm, while, signal of pyruvate groups methyl protons overlapped with signal of methylene backbone protons of PVI at δ = 2.2–2.5 ppm. Moreover, multiplet signals of alcoholic methine and methylene protons were shifted to δ = 2.8–3.2 ppm. ¹H NMR proved the synthesis of XG-g-PVI.

4.2.3. X-Ray diffraction (XRD) analysis

XRD patterns of investigated samples (XG and grafted XG (%G 444%)) are illustrated in Fig. 5. The results indicated that unmodified XG exhibited amorphous pattern (Pandey & Ramontja, 2016), while, XRD pattern of modified XG (XG-g-PVI) illustrated the semi crystalline structure. In XRD pattern of XG-g-PVI, small diffraction peaks along the pattern and large diffraction peaks at 2θ = 30°, 31° and 32° related to the semi crystallinity of PVI. From the XRD patterns, peak at 2θ = 17° corresponding to gauche-gauche (g−g) conformation was appeared in XG-g-PVI, while in XG, this peak was disappeared. These results were in agreement with the literature (Simione & Barbiero, 2006).
pattern of XG-g-PVI, it clarified that PVI was grafted onto XG chains and the grafted PVI had high ordered crystalline structure due to hydrogen bonding interaction between nitrogen atom in imidazole ring in PVI and hydroxyl (-OH) in XG chains (Scheme 2).

**Fig. 4.** $^1$H NMR of a) XG and b) XG-g-PVI.

**Fig. 5.** XRD of XG and XG-g-PVI.

**Scheme 2.** H-bonding interaction of hydroxyl (-OH) groups of XG chains and nitrogen atom in imidazole ring of PVI in XG-g-PVI copolymer.
showed enormous changes on the surface of biopolymer particles and these changes can be easily seen in FE-SEM images. The surface of XG showed irregular morphology as lobules (Pandey & Ramontja, 2016) that was changed to smooth surface in copolymer sample. Consequently, the surface morphology supported the grafting of PVI on to the XG chains.

4.2.6. Elemental analysis

Structure of grafted XG with PVI was proved with elemental analysis and the results showed that% of C, H, O and N in unmodified XG were 37.77%, 6.37%, 55.23% and 0.63% respectively. Appearance of nitrogen atom in analysis of XG (as traces) might be because of presence of impurities such as proteins from XG production during fermentation process (Mendes et al., 2011). While, results of elemental analysis of XG-g-PVI (%XG 444%) showed that% of C, H, O and N were 28.40%, 3.13%, 57.04% and 11.43%, respectively. These results showed a decrease in% C, % H and% O as a result of the dilution effect made by grafting reaction with PVI. On the other hand, the presence of Nitrogen (N = 11.43%) in the XG-g-PVI gave an additional proof for the grafting process. These results were similar to literature (Maity & Sa, 2014; Kaity & Ghosh, 2013).

4.3. Antibacterial activity test

Antibacterial activity of XG and different grafting percentage of XG with PVI was investigated against two pathogenic bacteria that cause food borne diseases namely S. aureus (as Gram-positive bacterium), and E. coli (as Gram-negative bacterium) using the well diffusion method. The antibacterial activity results of tested samples are tabulated in Table 1. There is no antibacterial activity for XG against the two pathogenic tested bacteria, this confirmed that XG is microbially attacked (Kumar et al., 2009), while, results of

![TGA Curves for XG and XG-g-PVI](image)

**Fig. 6.** TGA Curves for XG and XG-g-PVI.

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<th>Samples</th>
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<td>Gram- negative bacteria</td>
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<td></td>
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<tr>
<td>Non-modified XG</td>
<td>–ve</td>
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<tr>
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<td>13.0 ± 0.5</td>
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4.2.5. Surface morphology analysis

FE-SEM images of XG and XG-g-PVI with a magnification of 1000 are shown in Fig. 7. The images revealed some variations in surface morphology of XG and its grafted sample. The grafted sample

![FE-SEM images of a) XG and b) XG-g-PVI (X 1000)](image)

**Fig. 7.** FE-SEM images of a) XG and b) XG-g-PVI (X 1000).
grafted XG samples exhibited an increase in antibacterial activity with increasing of grafting% due to the increase PVI content on XG chains. Several mechanisms are elucidating the antibacterial activity of PVI have been postulated (Sharma, Wilson, & Dubey, 2016), the mechanisms are based on:

I) The electrostatic interactions between positive charge of vinyl imidazole in PVI and negative charge on cytoplasmic membrane of bacterial cell surface.

II) Hydrophobic interactions between the imidazole moiety in PVI and the bacterial cytoplasmic membrane which leads to the disruption of the bacterial membranes and death of bacterial cells.

III) Penetration of imidazole ring into the bacterial cell membrane and binds to DNA resulting in its destabilization because of mutation that changes the protein sequences and leads to the damage of DNA.

5. Conclusion

Grafted xanthan gum (XG) with poly(N-vinyl imidazole),PVI, was synthesized successfully by free radical polymerization mechanism and its proposed structure was proven by elemental analysis, FTIR and $^1$H NMR techniques. The crystallinity of XG-g-PVI was exhibited with XRD technique and the results showed the change of the amorphous XG to semicrystalline grafted XG due to H-bonding interactions between PVI and XG chains. TGA analysis illustrated that modified XG was more thermally stable than unmodified XG at elevated temperatures. On the other hand, images of FE-SEM illustrated a change in surface morphology from irregular shape as lobules in XG to smooth surface in XG-g-PVI. The antibacterial activity of tested samples (XG and grafted samples with different%XG) was done against two pathogenic bacteria (E. coli and S. aureus) and the results showed that XG has no antibacterial activity, while, grafted XG copolymers have antibacterial activity and the activity increased with the increase in the percent graft.

References


