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Synthesis, characterization and antibacterial activity of poly(*N*-vinylimidazole) grafted chitosan

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Abstract

Poly(*N*-vinylimidazole) has been grafted onto chitosan in dilute acetic acid solution via ceric ion initiation. The effect of the amounts of *N*-vinylimidazole monomer, cerium(IV) ammonium nitrate initiator, chitosan substrate, as well as chitosan molecular weight, temperature and reaction time on the grafting yield has been investigated. The maximum grafting yield achieved was 140% in 1.0% (w/V) chitosan solution at 0.2 M *N*-vinylimidazole, 70 °C within 3 h under nitrogen atmosphere. The grafted products were characterized by gravimetric methods, FTIR and DSC. Grafted chitosans with lower grafting yields less than 108% were completely soluble in neutral water. Products with higher % grafting values swell in aqueous solution. Grafted products have improved antibacterial activity.

Keywords: 1-Vinylimidazole; CAN; Cerium; Enzymes; Water-soluble polymers

1. Introduction

Chitosan [poly(β-(1 → 4)-2-amino-2-deoxy-D-glucose)] is the *N*-deacetylated derivative of chitin, a very abundant polysaccharide, found naturally in the exoskeletons of insects, shells of crustaceans, and in fungal walls. Chitosan is a functional biomaterial with numerous industrial, pharmaceutical and medical applications (Dutta, Ravikumar, & Dutta, 2002). Chitosan films, fibers, micro and nanoparticles have been reported for tissue engineering, drug, vaccine and DNA delivery applications (Yilmaz, 2004). Chitosan acts as a polycation in aqueous acid solutions like acetic acid and hydrochloric acid via protonation of amine functions. Chitosan derivatives soluble in neutral and in basic media in addition to aqueous acid solutions are needed as biomaterials (Sashiwa & Aiba, 2004). For example, water soluble derivatives promise to be useful as antibacterial agents. Xie, Xu, Wang, and Liu (2002) prepared water soluble hydroxypropyl chitosan with antibacterial activity via grafting by maleic acid sodium. Qu, Wirsen, and Albertsson (1999) grafted D,L-lactic acid onto chitosan to obtain pH sensitive polymer gels for gastrointestinal-specific drug delivery. Synthesis of O-PEGylated chitosans soluble in aqueous medium in a wide pH range have been studied by Gorochovceva and Makuska (2004). Chitosan grafted with poly(*N*-isopropylacrylamide) (Lee, Jung, Park, Park, & Ryu, 2004) and poly(dimethylamino)ethyl methacrylate (Liang, Ni, Zhang, & Yu, 2004) have been reported to be water soluble products. Water solubility under basic conditions has been achieved by enzymatic grafting of a natural product onto chitosan (Kumar, Smith, & Payne, 1999). It has been demonstrated by our group that solubility of the grafted products is controlled by the extent of grafting. Grafting of maleic acid onto chitosan yields a range of products from fully water soluble to hydrogels (Hasipoglu, Yilmaz, Yilmaz, & Caner, 2005).

Similar to all other vinyl monomers, vinylimidazoles readily undergo free radical homopolymerization in organic medium to form high molecular weight polymers. Poly(*N*-vinylimidazole), a well known water soluble polymer, was applied to modify various natural or synthetic

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polymers through grafting. Preparation of poly(vinyl alcohol) membranes grafted with *N*-vinylimidazole/acrylic acid binary monomers is a recent study (Ajji & Ali, 2005). Poly(*N*-vinylimidazole) grafted magnetic nanoparticles were also studied (Takafuji, Ide, Ihara, & Xu, 2004). Electrochemistry of *N*-vinylimidazole grafting has also been studied (Bojanic, Jovanovic, Tabakovic, & Tabakovic, 1996; Wang, Vora, Kang, & Neoh, 2004).

Polymers containing the imidazole ring or its derivatives are known to be useful biomaterials since they show antibacterial activity and have improved biodegradability. Saravanan, Selvan, Gopal, Gupta, and De (2005) studied the antibacterial activity of some imidazole-5-(4H)one derivatives. Soykan, Coskun, and Delibas (2005) used copolymers of *N*-vinylimidazole with phenacyl methacrylate for microbial screening. Most recently, Chauhan, Singh, Chauhan, Dhiman, and Kumar (2006) grafted cellulose with *N*-vinylimidazole for use as supports in enzyme immobilization. Improved biodegradability of imidazole polymers was also recorded (Allcock, 1999).

Pekel's group studied various other biological and metal chelating properties of poly(*N*-vinylimidazole) and its hydrogels (Pekel & Guven, 1999, 2003; Pekel, Sahiner, & Guven, 2000, 2001, 2002, 2003; Salih, Pekel, & Guven, 2001). Metal ion binding properties of poly(*N*-vinylimidazole) hydrogels have been also studied by Rivas, Maturana, Molina, Gomez-Anton, and Pierola (1998).

Chitosan and imidazole containing polymers have some common properties compatible with biomedical applications. They act as antibacterial agents, have metal binding properties and are biocompatible and biodegradable. The advantage of *N*-vinylimidazole as a grafting agent is its water solubility. Grafting it onto chitosan would lead to products with improved chemical, physical and biological properties. Therefore the purpose of this study is twofold; to improve the water solubility of chitosan and use the natural/synthetic hybrid products in some biochemical applications, namely antibacterial activity.

Thus, in this study, grafting poly(*N*-vinylimidazole) onto chitosan, the optimum grafting conditions, characteristics of the products, and antibacterial activity results have been reported.

2. Experimental

2.1. Materials

Two different chitosan samples C85 (a product of Aldrich with 85% degree of deacetylation and M_v of 2.0×10^6) and C90 (a product of Primex with 90% degree of deacetylation and M_v of 3.0×10^5) were used. *N*-Vinylimidazole (NVI) (Merck) was vacuum distilled before use. Cerium ammonium nitrate (CAN) (Aldrich) was used without any purification. Acetic acid, hydrochloric acid, nitric acid (Merck), hydrobromic acid (BDH) and sodium hydroxide (Merck) were used as supplied. Acetone, ethanol, dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) were all products of Merck and were used without any purification.

The standard bacteria *Staphylococcus aureus* (*S. aureus*) ATCC6538P and *Bacillus subtilis* (*B. subtilis*) ATCC6633, and the bacteria produced under laboratory conditions *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*), which were supplied from the State Laboratory-Lefkosa, were used in antibacterial studies.

2.2. Grafting of NVI onto chitosan

Given amounts of chitosan were dissolved in 1% acetic acid and was stirred overnight followed with 30 min purging with nitrogen gas. The monomer (NVI) and the initiator (CAN) were added under nitrogen atmosphere and the reaction was carried out at constant temperature by stirring at 50 rpm. The resultant solution was poured into acetone with vigorous stirring for precipitation. The product was removed, washed and extracted extensively for 48 h with ethanol for homopolymer removal followed by drying at 60 °C under vacuum. Percent grafting (%G) and percent efficiency (%E) were determined as follows:

$$\%G = \frac{W_2 - W_1}{W_1} \times 100 \quad (1)$$

$$\%E = \frac{W_2 - W_1}{W_3} \times 100 \quad (2)$$

where W_1, W_2 and W_3 are weight of chitosan, grafted chitosan and NVI, respectively.

The effect of the monomer concentration, the initiator concentration, chitosan concentration and the reaction time on grafting was studied at 70 °C. The effect of temperature on grafting was also followed at the range of 30–80 °C. Each experiment was carried out twice. The results agreed with each other within 10% error.

2.3. Determination of degree of deacetylation of chitosan

The degree of deacetylation (DD) of used chitosan samples was determined by both titrimetric and infrared spectroscopic methods (Domszy & Roberts, 1985). Comparable DD values have been obtained by both methods. An average of the two values was taken as 85% for C85 and as 90% for C90.

2.4. Determination of molar mass of chitosan

Viscosity average molecular weight of polymers were determined at 30 °C in a 0.1 M acetic acid/0.2 M sodium acetate buffer solution using the relationships proposed by Wang, Bo, Li, and Qin (1991).

$$[\eta] = 1.424\times10^{-3}\mathrm{M}^{0.96}(\mathrm{mL/g})(\text{for C85}) \quad (3)$$

$$[\eta] = 6.589\times10^{-3}\mathrm{M}^{0.88}(\mathrm{mL/g})(\text{for C90}) \quad (4)$$

$\overline{M_v}$ for C85 and C90 was calculated as 2.0×10^6 and 3.0×10^5, respectively.

2.5. Instrumentation

FTIR analysis was made by using a Mattson 5000 Satellite FTIR Spectrometer. Differential scanning calorimetry (DSC) measurements were carried out using a General V4.1C DuPont 2000 Calorimeter with a heating rate of 10 °C/min in air.

2.6. Solubility tests

The grafted products were tested for solubility in water, 1% acetic acid, ethanol, 1% acetic acid:ethanol mixture (1:1), glacial acetic acid:ethanol mixtures (1:1 and 1:2), DMF, DMSO and THF. The solubility of the products was also tested in HCl solutions of pH 1, 3, 5 and 7. In each case, 1% w/V solution was used.

2.7. Antibacterial activity tests

The antibacterial tests were carried out by standard disc-agar diffusion method by using 6 mm diameters discs prepared from Whatman-4 filter paper, and measuring the inhibition zones (mm).

3. Results and discussion

3.1. Influence of reaction conditions on the extent of grafting

3.1.1. Effect of NVI concentration

The effect of NVI concentration on the grafting yield has been studied gravimetrically by using two types of chitosan samples C85 (degree of deacetylation = 85%, molar mass = 2.0×0^6) and C90 (degree of deacetylation = 90%, molar mass = 3.0×0^5). The results are shown as percent grafting and percent efficiency in Fig. 1 which indicates clearly that grafting of PNVI onto chitosan has been achieved. For both chitosan samples percent grafting increases with increasing NVI concentration to a maximum of 140% at around 0.2 M for C85 and 0.3 M for C90 and then reaches a plateau with a slight tendency to decrease, showing that higher NVI concentrations do not promote further grafting. This can be explained by degradative chain transfer to NVI as Bamford and Schofield (1981) proposed. The following scheme (Scheme 1) represents the radical chain transfer mechanism.

Degradative chain transfer generates unreactive species with a low tendency for propagation, thus, retarding further grafting. Another reason for the observed behavior could be the substantial amount of polymer grafted onto chitosan backbone, which creates steric hindrance for further grafting.

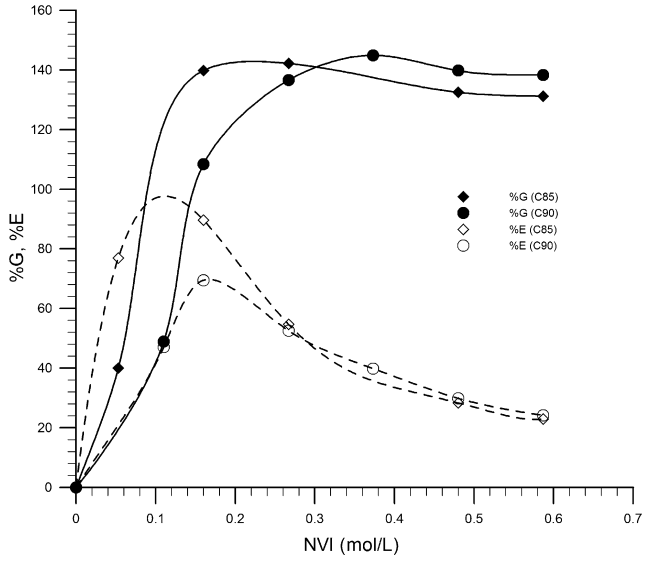


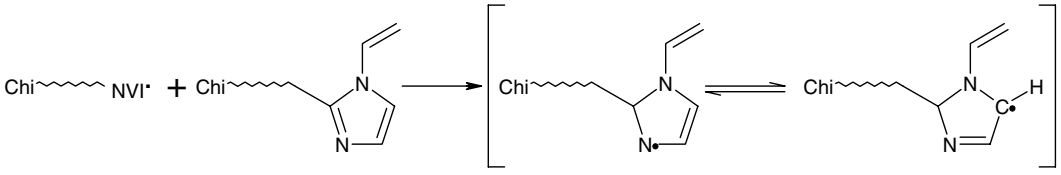
Fig. 1. Effect of NVI concentration on grafting to chitosan (C85 and C90). Reaction conditions: 0.2 g chitosan; 0.7 g CAN; 180 min; 70 °C; solvent: 20 mL 1% acetic acid.

3.1.2. Influence of chitosan molecular weight and degree of deacetylation

Fig. 1 also reveals that the molecular weight and degree of deacetylation of chitosan does not have a significant effect on the maximum grafting yield. The slight variations in the extent of grafting with monomer concentration for two different samples (C85 and C90) have been attributed to the heterogenities in the microstructures of the chitosan substrates. Somewhat lower grafting percentages obtained with the higher molecular weight chitosan should be due to the increased viscosity of the solution restricting the diffusion of the species in solution.

3.1.3. Influence of chitosan concentration

The influence of chitosan (C90) concentration on % G and % E where all other variables were kept constant are shown in Fig. 2. Grafting behavior of NVI on chitosan follows a trend such that grafting yield reaches a maximum value, 108% (meaning $n_{NVI}:n_{chi} = 1.8:1$), at a chitosan concentration of 1.0% (w/V). Further increases in the concentration of chitosan solution, result in substantially lower grafting yield values. Although the number of sites



Scheme 1.

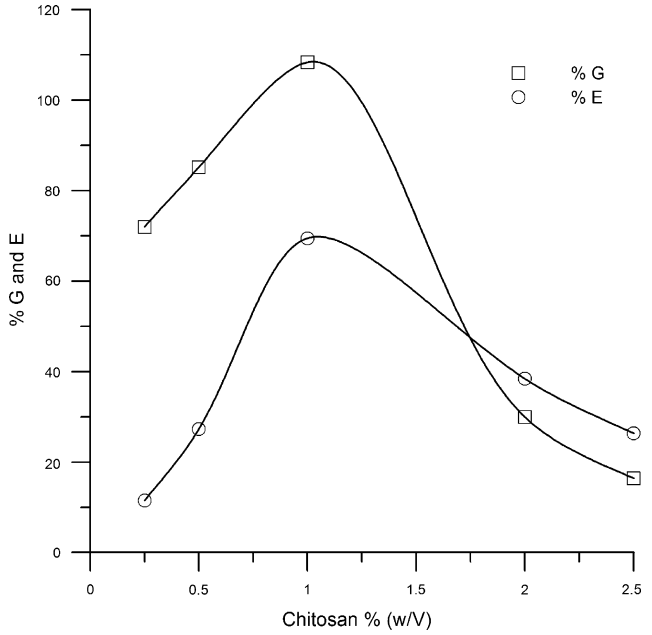


Fig. 2. Effect of chitosan amount. Reaction conditions: 0.7 g CAN; 0.312 g (0.16 M) NVI; 180 min.; 70 °C; solvent: 20 mL 1% acetic acid.

susceptible to oxidation increases with the amount of chitosan, the amount of the initiator limits the number of free radical sites available for grafting. The fact that the grafting yield decreases rather than leveling off with increasing chitosan concentration, may be attributed to the increased viscosity of the solution with higher polymer concentration which restricts the number of effective collisions.

3.1.4. Effect of initiator concentration

Fig. 3 shows the dependence of the grafting yield on the initiator (cerium ammonium nitrate, CAN) concentration. Both percent grafting and percent efficiency values increase with increasing ceric ion concentration until it reaches a maximum value of around 108% at 0.26 M of CAN. A further increase in CAN concentration is accompanied by a considerable decrease in the grafting yields. This could be due to several known reasons such as the competition between initiation and termination reactions. However, the most obvious reason for this is the pH effect. Fig. 3 shows that the initial pH of the grafting medium decreases with increasing CAN concentration. Protonation of NVI at second nitrogen as shown in Scheme 2 gives rise to a resonance stabilized system. Degradative chain transfer to monomer is suppressed since the resonance stabilization energy would be lost on formation of a radical on the vinyl carbon adjacent to N. Hence, higher grafting yields are obtained under more acidic conditions (Bamford & Schofield, 1981). A further increase in the acidity of the medium is accompanied by a decrease in percent grafting values which may be attributed to an abundance of hydrogen protons acting as free radical terminators.

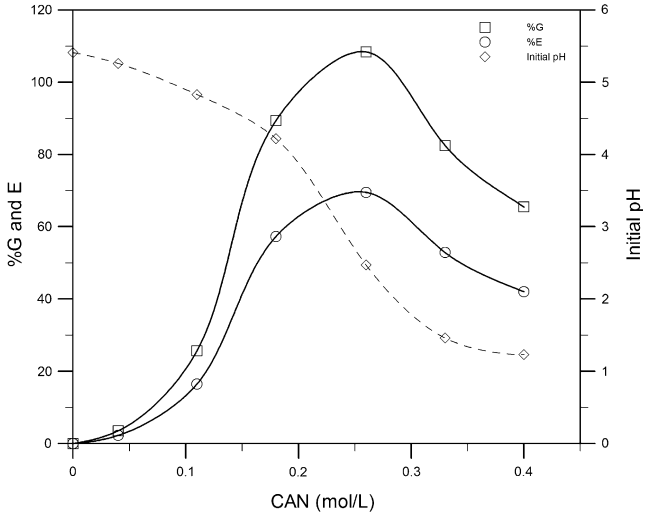
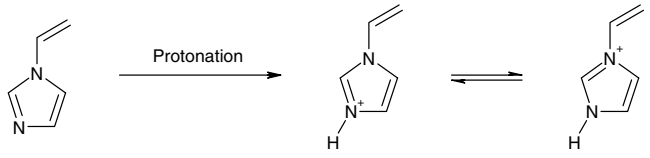


Fig. 3. Effect of initiator concentration. Reaction conditions: 0.2 g chitosan (C90); 0.312 g (0.16 M) NVI; 180 min.; 70 °C; solvent: 20 mL 1% acetic acid.

3.1.5. Influence of reaction temperature

The effect of temperature on percentage of grafting as well as grafting efficiency is shown in Fig. 4. It is apparent that the optimum temperature range for maximum grafting is 60–70 °C. Further increase in temperature somewhat reduces the percentage of grafting. A similar trend was observed for percent efficiency. At higher temperatures



Scheme 2.

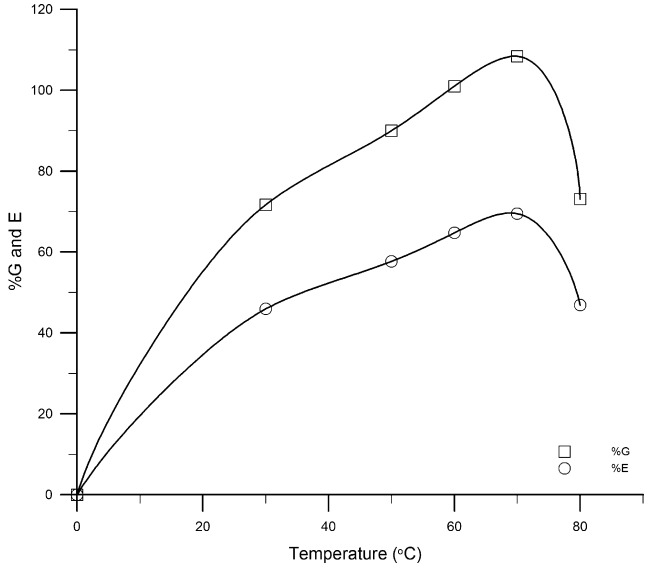


Fig. 4. Effect of reaction temperature. Reaction conditions: 0.2 g chitosan (C90); 0.312 g (0.16 M) NVI; 180 min; solvent: 20 mL 1% acetic acid.

chain transfer reactions are favored decreasing the extent of grafting. Also inefficient activity of CAN at elevated temperatures would give rise to less efficient initiation causing a decrease in grafting. Consequently, the combined factors of less efficient initiation and increased rate of termination accounts for the decreasing amount of graft copolymer.

3.1.6. Effect of reaction time

The influence of reaction time on grafting is shown in Fig. 5. Increasing the reaction time produces an increase in grafting. At longer reaction times, the percentage of grafting begins to level off. The percentage of grafting reaches a plateau after 3 h due to the depletion of the monomer, initiator and available grafting sites as the reaction proceeds. Grafting efficiency also shows a similar trend.

3.2. Characterization of the products

3.2.1. Thermal analysis

The DSC curves of chitosan (C90) and the grafted products with 49% and 145% grafting values are shown in Fig. 6a–c, respectively. Initial decomposition takes place at 308 °C for chitosan, 275 °C for (b) and 265 °C for (c). Additional decomposition peaks appear at 235 °C as a shoulder and 210 °C for (b) and at 216 and 196 °C for (c). These peaks indicate a structural change in chitosan chains which is due to the grafting of PNVI chains. The grafted PNVI chains on chitosan backbone may result in the breaking of the intermolecular hydrogen bonds between chitosan chains. This result is consistent with improved solubility of grafted products in water which will be discussed later. The observed shift in the initial decomposition temperature and the increase in number of additional peaks at lower temperatures occur parallel to the increase in percent grafting. The glass transition temperature of PNVI could not be observed in the DSC curves. This might be an indication that the grafted PNVI chains on the chitosan backbone are oligomers.

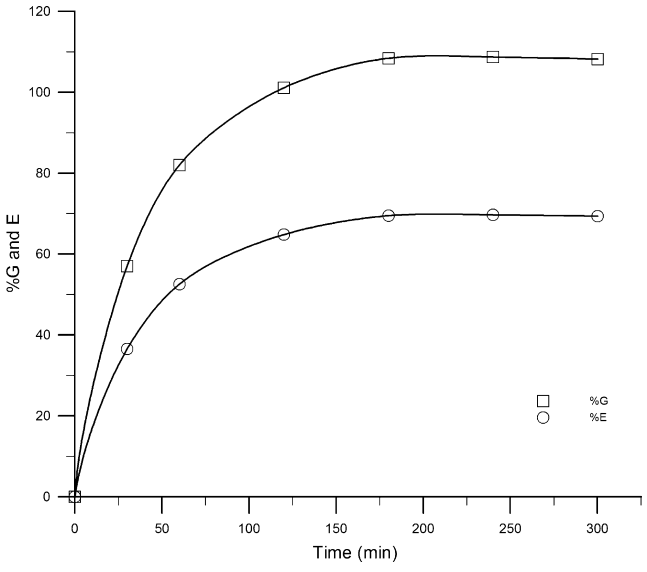


Fig. 5. Effect of reaction time. Reaction conditions: 0.2 g chitosan (C90); 0.312 g (0.16 M) NVI; solvent: 20 mL 1% acetic acid.

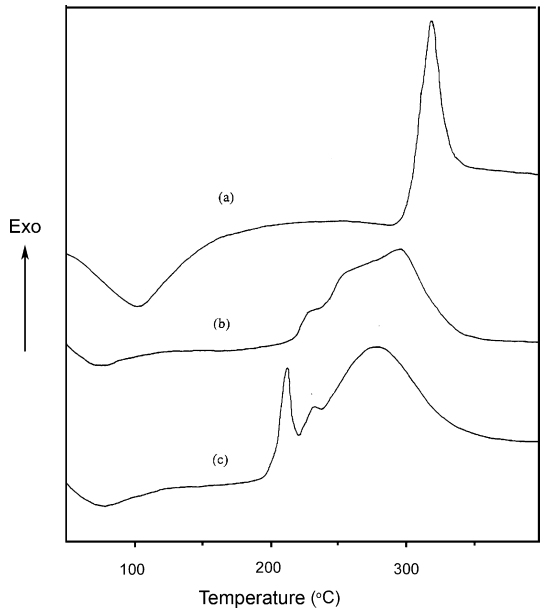


Fig. 6. DSC curve of (a) pure chitosan (C90), (b) 49% grafted chitosan, and (c) 145% grafted chitosan.

3.2.2. Fourier transform infrared analysis FTIR

The superimposed FTIR spectra of chitosan (C90) and 145% grafted copolymer are shown in Fig. 7. Amide I and II bands of chitosan are lost and two new bands appear at 1633 and 1527 cm^{-1} due to C=C and C=N stretching vibrations of imidazole ring (Hummel & Scholl, 1990) as shown in Scheme 3. Again peaks in the range 1475–1250 cm^{-1} that overlap in an enhanced form are assigned to various bending vibrations of C–H bonds and C–N vibrations of both chitosan and PNVI. Besides C–H bond vibration of imidazole ring is also observable at around 826 cm^{-1}. Therefore, it can be deduced that NVI incorporated onto chitosan backbone.

FTIR spectral study of products with increasing degree of grafting revealed that the intensities of C=C, C=N and C–H vibration bands of imidazole ring increase as grafting degree of PNVI on to chitosan backbone increase.

3.3. Solubility tests

Water solubility of the grafted products are summarized in Table 1. Water soluble products have been obtained at 49% and 108% grafting values. Hence, grafting of PNVI onto chitosan produced a novel water soluble chitosan as predicted. Water soluble products are of practical value as the lack of solubility of chitosan in neutral water limits its useful applications. As NVI is a water soluble monomer which is converted by free radicalic polymerization into a water-soluble polymer, it imparts this property to the

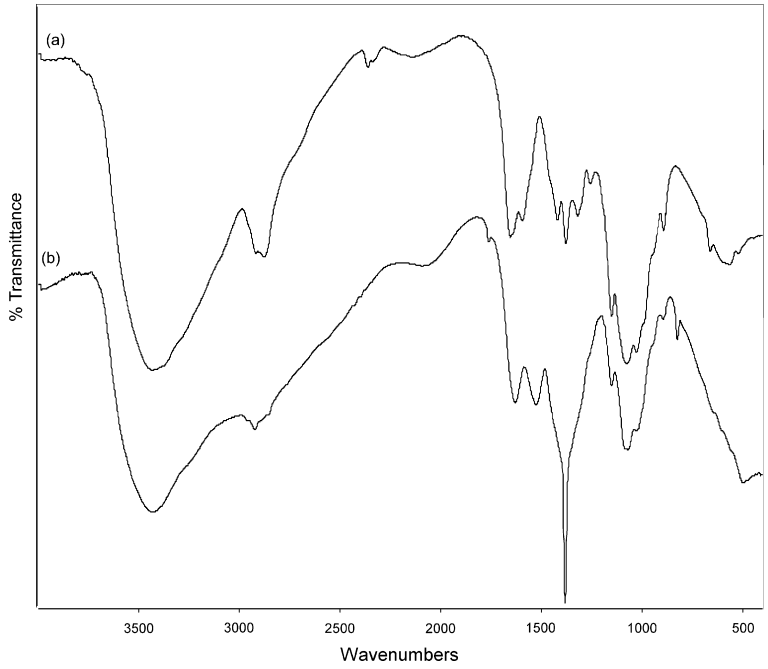
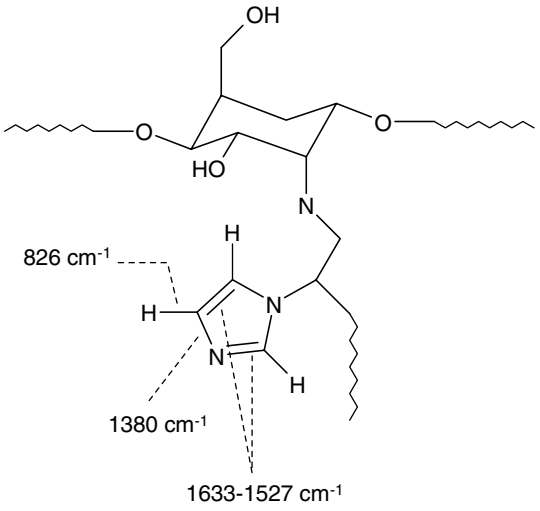


Fig. 7. FTIR spectra of (a) chitosan (C90) and (b) 145% grafted copolymer.



Scheme 3.

Table 1
Results of solubility tests on 49% grafted chitosan

Solvent	Observation
1% Acetic acid	Soluble
Ethanol	Insoluble
1% Acetic acid:ethanol (1:1)	Cloudiness
Glacial acetic acid:ethanol (1:1)	Swelling
Glacial acetic acid:ethanol (1:2)	Swelling
DMF	Insoluble
DMSO	Insoluble
THF	Insoluble
Distilled water	Soluble

substrate to a certain extent. As revealed by spectroscopic and thermal analyses, grafting proceeded via acetamido and amine groups leading to partial disruption intermolecular hydrogen bonding. Hence, the combined factors, the water soluble nature of PNVI and reduced hydrogen bonding improved the solubility of chitosan in pure water. There is a correlation between percent grafting and solubility in water as shown in Table 2. While the samples with percent grafting values up to 108% are soluble in water, higher grafting results in insoluble but highly swollen polymers. This behaviour may be attributed to a slight crosslinking at higher grafting yields.

Although PNVI is soluble in organic solvents such as ethanol, DMF, DMSO and THF, grafted chitosans were found to be insoluble in these solvents. This shows that chitosan's nature is predominant over PNVI in these solvents for samples studied.

3.4. The antibacterial activity of chitosan-g-PNVI copolymers

A preliminary study has been carried out to compare the antibacterial activity of grafted chitosan hydrochloride film samples with that of chitosan. The study was carried out

Table 2
Effect of percent grafting on solubility

%G	Solubility
49	Soluble
108	Soluble
137	Swelled
145	Swelled
140	Swelled
138	Swelled

Table 3
Inhibition zone tests for chitosan and *N*-vinylimidazole grafted chitosan samples

Sample, % grafting	Average film weight (μg)	Inhibition zone (mm)			
		Gram-negative		Gram-positive	
		P. aeruginosa	*E. coli*	*B. subtilis*	*S. aureus*
Chitosan (C90)	134	7 ± 0.2	6 ± 0.4	8 ± 0.0	8 ± 0.0
82.5% graft	134	9 ± 0.0	8 ± 0.6	10 ± 0.0	11 ± 0.6
145% graft	134	11 ± 0.0	10 ± 0.4	13 ± 0.2	12 ± 0.6
Chitosan (C90)	296	10 ± 0.0	9 ± 0.6	10 ± 0.0	11 ± 0.0
82.5% graft	296	12 ± 0.0	13 ± 0.4	15 ± 0.4	14 ± 0.4
145% graft	296	14 ± 0.6	14 ± 0.6	17 ± 0.0	16 ± 0.6

against *P. aeruginosa*, *E. coli*, *B. subtilis* and *S. aureus* using the inhibition zone method. The results are shown in Table 3. It was observed that grafting of NVI improved the antibacterial activity of chitosans. While the inhibition zone diameter for chitosan film ranged between 9 and 11 mm against indicated bacteria, the inhibition zone increased up to 17 mm (against *B. subtilis*) by grafting.

Although the difference is not significant, activity of gram-positive bacteria seems to be more pronounced; increase in the inhibition zone diameter is 4–5 mm in gram-negative ones whereas it is 5–7 mm in gram-positive ones.

Grafted samples showed an increasing antibacterial activity as the degree of grafting increased for all of gram-negative and gram-positive bacterias; a minimum of 2 mm increase was observed consistently when the grafting percentage increased from 82.5% to 145%.

Average film weight (thickness) also effected the degree of antimicrobial activity of both chitosan and grafted chitosan samples. An 3–4 mm increase was observed when the average film weight was increased from 134 to 296 μg.

4. Conclusions

PNVI has been grafted onto chitosan by ceric ion initiation in aqueous acidic solution. The maximum grafting yield was obtained as 140% for 85% deacetylated chitosan in 1% (w/V) chitosan at 0.2 M NVI, 70 °C, 3 h reaction time using 1% (w/V) acetic acid as the solvent.

Grafting yield increases with the amount of monomer, NVI, reaching a plateau at 0.2 M. Grafting yield increases with increasing amount of chitosan, the initiator, CAN, temperature and time showing a similar tendency in all four cases, following a decrease after reaching a maximum. There is a negligible effect of chitosan molecular weight on the grafting yield within the high molecular weight range studied (3.0×105–2.0×106).

Grafting of PNVI onto chitosan improves water solubility of chitosan and the solubility of the products is controlled by the extent of grafting. Chitosan-g-PNVI products with lower grafting values (<108%) are soluble in neutral water while raw chitosan is not. Increasing grafting yield decreases the water-solubility.

Grafting products show improved antibacterial activity. The activity increases with increasing percent grafting and film thickness.

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