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Synthesis, characterization and antimicrobial activity of poly (*N*-vinyl imidazole) grafted carboxymethyl chitosan

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ABSTRACT

Poly (*N*-vinyl imidazole) (PNVI) has been grafted onto carboxymethyl chitosan in aqueous solution using potassium persulphate (KPS) as initiator. The effect of the monomer and initiator concentration, the reaction temperature and time on the grafting yield have been investigated. The maximum grafting yield was achieved at [KPS] = 8×10^{-2} mol/L, [M] = 1 mol/L at reaction temperature = 60 °C within reaction time = 2.5 h. The grafted products were characterized by FTIR, elemental analysis, SEM photographs, solubility tests, thermal analysis and antibacterial activity. Grafted products have improved the antimicrobial activity of carboxymethyl chitosan.

1. Introduction

Chitosan [Poly (β-(1→4)-2-amino-2-deoxy-D-glucan] is *N*-deacetylated derivative of chitin extracted from the shells of crustaceans like crabs and shrimps. It is obtained through the alkaline hydrolysis of chitin to convert the acetamide group into amino group. Chitosan is a biocompatible polymer and has found many applications as biomaterials in tissue engineering and in controlled drug release systems for various routes of delivery (Kumar, 2000; Mphahlele, 2005; Peng & Zhang, 2009; Rinaudo, 2006; Xie, Xu, Wang, & Liu, 2002a, 2002b). Chemical modification of Chitosan is an important topic for production of multifunctional material. Graft copolymerization of vinyl monomers onto chitosan and other natural polymers can introduce desired properties and enlarge the field of their potential applications (El-Sherbiny, Lins, Abdel-Bary, & Harding, 2005; Jigar & Sinha, 2007; Joshi & Sinha, 2006).

Recently, ceric ion initiated graft copolymerization of *N*-vinyl imidazole (NVI) onto chitosan was reported (Hamit, Elvan, & Osman, 2007). The maximum grafting yield achieved was 140% in 1.0% (w/v) chitosan solution at 0.2 M *N*-vinyl imidazole at 70 °C within 3 h under nitrogen atmosphere. Chitosan derivatives soluble in neutral and in basic media in addition to acidic solutions are needed as biomaterials (Sashiwa & Aiba, 2004). Water soluble hydroxyl propyl chitosan was prepared (Xie et al., 2002a, 2002b) with antibacterial activity via grafting by sodium maleate.

Similar to all other vinyl monomers, vinyl imidazoles readily undergo free radical polymerization in water to form high molecular weight water soluble polymers (PNVI). Polymers containing the imidazole ring or its derivatives are known to be useful biomaterials since they show antibacterial activity and have improved biodegradability. The antibacterial activity of some imidazole-5-(4H)-one derivatives was studied (Saravanan, Selvan, Gopal, & Gupta, 2005). Chitosan and imidazole containing polymers have some common properties useful for biomedical applications. They act as antibacterial agents, have metal binding properties and are biocompatible and biodegradable. The advantage of PNVI as a grafting agent is its water solubility.

Wenming et al. (Tao, Peixin, Qing, Jian, & Wenming, 2003) had prepared carboxymethyl chitosan and characterized it by FTIR, ^{1}H NMR and elemental analysis. Graft copolymerization of methacrylic acid (MAA) onto carboxymethyl chitosan was done using ammonium persulfate (APS) as an initiator and was carried out in an aqueous solution. Evidence of grafting was obtained by comparison of FTIR spectra of carboxymethyl chitosan and the grafted copolymer as well as solubility characteristics of the products. The optimum conditions were reported.

O-Carboxymethyl chitosan was prepared and characterized by FTIR spectroscopy and X-ray diffraction (Jigar & Sinha, 2007). Grafting of Acrylamide (Am) onto carboxymethyl chitosan using ceric ammonium nitrate (CAN) as an initiator was carried out under nitrogen atmosphere in aqueous solution. Optimum grafting conditions were mentioned.

Carboxymethyl chitosan was prepared (El-Sherbiny, 2009) and characterized by FTIR spectroscopy, elemental analysis and X-ray

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diffraction. Graft copolymerization of *N*-acryloylglycine (NAGly) onto carboxymethyl chitosan using 2,2-dimethoxy-2-phenyl acetophenone (PI) as photoinitiator was carried out under nitrogen atmosphere in aqueous solution. The effects of concentration of NAGly, PI and reaction time on the extent of grafting were investigated by determining the grafting percentage and grafting efficiency.

In the present study, the various parameters affecting the grafting of PNVI onto carboxymethyl chitosan, characterization of the grafted products and their antibacterial activity results will be reported.

2. Experimental

2.1. Materials

Chitosan (with a degree of deacetylation 88%) was purchased from Funakoshi Co. Ltd., Japan. Potassium persulfate (KPS) was supplied from S.D. Fine Chemical, India.

N-Vinylimidazole (NVI) was supplied by Merck (Schuchardt OHG, Hohenbrunn, Germany). Monochloro acetic acid was obtained from LOBA chemie pvt. Ltd., Mumbai, India. Other reagents and solvents were of analytical grades and were used as received.

2.2. Instrumentals

2.2.1. Infrared spectroscopy

FTIR spectra were recorded on Testcan Shimadzu IR-Spectrophotometer (model 8000) within the wave number range of 4000–600 cm^{-1} at 25 °C.

2.2.2. Scanning electron microscopy (SEM)

The dry sample, spread on a double sided conducting adhesive tape, pasted on a metallic stub, was coated with a gold layer of 100 μm thickness using an ion sputter coating unit (JEOL S150A) for 2 min and observed in a JEOL-JXA-840A Electron probe micro analyzer at 20 kV.

2.2.3. Thermogravimetric analysis

Thermogravimetric analysis was done on TGA-50H Shimadzu Thermogravimetric Analyzer. Samples were heated from 0 to 500 °C in a platinum pan with a heating rate of 10 °C/min under N_2 atmosphere with flow rate of 25 ml/min.

2.3. Experimental techniques

2.3.1. Preparation of carboxymethyl chitosan (CMCh)

Carboxymethyl chitosan (CMCh) was prepared according to the method described in literature (Chen & Park, 2003) via stirring 5 g of chitosan in 100 ml of 20% NaOH (w/v) for 15 min. Then 15 g of monochloro acetic acid was added portion wisely to the reaction medium and stirring was continued for 2 h at 40 °C. The reaction mixture was then neutralized with 10% acetic acid, poured into an excess of 70% methanol, filtered using a G_2 sintered glass funnel and washed with methanol. The produced CMCh sodium salt was dried in a vacuum oven at 55 °C for 8 h. The sodium salt of carboxymethyl chitosan was then acidified with a methanol solution containing nitric acid; the excess acid was then removed by washing with a methanol–water solution till acid free. Dry the material. The degree of substitution (0.75) was determined according to the method described in literature (Wu, Chan, & Szeto, 2003).

2.3.2. Graft copolymerization

CMCh (0.5 g) dissolved in 25 ml distilled water was stirred for an hour followed with 30 min purging with nitrogen gas. A predetermined amount of *N*-vinyl imidazole monomer (NVI) was charged under nitrogen atmosphere and heated at 60 °C with continuous stirring for 15 min. After that, the required amount of initiator (KPS) dissolved in distilled water was added portionwise to the flask to initiate graft copolymerization. The reaction was continued for a predetermined temperature and time.

The grafting mixture being homogeneous (due to the presence of –COOH groups in CMCh) is precipitated with vigorous stirring in cold acetone. Homopolymer was extracted from the crude grafted material with ethanol using soxhlet apparatus for 8 h. The grafted samples were dried in an air oven at 60 °C till constant weight.

The grafting parameters were calculated according to the following equations (Kim, Ha Ch, & Jo, 2002):

$$\text{Graft yield } (\%G) = [(W_1 - W_0)/W_0] \times 100$$

$$\text{Homopolymer } (\%H) = [(W_2 - W_1)/W_3] \times 100$$

$$\text{Grafting efficiency } (\%GE) = [(W_1 - W_0)/(W_2 - W_0)] \times 100$$

where;

W_0, W_1 = the weights of the initial matrix and grafted matrix (i.e. wt. of grafted product after extraction), respectively.

W_2 = the crude product before extraction.

W_3 = the weight of monomer.

2.3.3. Solubility tests

The grafted products were tested for solubility in water, 1% acetic acid, 1% acetic acid:ethanol mixture (1:1), DMF, 1,4-Dioxane and THF. In each case, 1% (w/v) solution was used.

2.3.4. Antimicrobial activity tests for PNVI-g-CMCh copolymers

All procedures were performed under aseptic conditions.

2.3.4.1. Antibacterial assays. The method of (Jiang, Yu, & Chen, 2005) was adopted. One loopful of fresh bacteria (*Staphylococcus aureus* or *Escherichia coli*) was suspended in an appropriate amount of sterilized saline solution, forming a bacterial cell suspension. The viable cell number in the suspension was controlled via the turbidity comparison method. This suspension was diluted to a prescribed cell concentration with sterilized distilled saline solution, thus preparing a bacterial cell suspension that was directly used for the antibacterial tests for the grafted copolymers. In each test, 20 ml of the bacterial suspension and 0.2 g (dry weight) of the tested polymer sample were placed in a sterilized glass container with a cotton stopper. The container was shaken for 2 h (150 rpm), 1 ml of this suspension system was pipetted out from the container and quickly mixed with sterilized saline and then decimal serial dilutions were prepared. The viable cell number in each of the polymer/bacterial suspension systems at the contact time was determined by conventional spread-plate method. Replicates were made and colonies were counted after 24 h. of incubation on Nutrient Agar medium at 37 °C. The percentage of inhibition was counted as follows:

$$\%\ \text{Inhibition} = \frac{\text{Untreated} - \text{Test polymer}}{\text{Untreated}} \times 100$$

2.3.4.2. Antifungal assays. *Fusarium oxysporum* and *Aspergillus fumigatus* were grown in modified Czapek-Dox medium containing (g L^{-1}): sucrose, 20; $NaNO_3$, 2; K_2HPO_4, 1; KCl, 0.5; $MgSO_4{\cdot}7H_2O$, 0.5; $FeSO_4{\cdot}7H_2O$, 0.01.

Cultivation was carried out in 250-Erlenmeyer flasks each containing 50 ml of the above medium. The polymers were tested at a concentration of 10 mg/ml. Inocula were prepared by harvesting spores from 7-day-old PDA slants of the tested fungi in sterilized distilled water containing 0.1% (v/v) Tween-80. The concentrations of spore suspensions were determined in a hemacytometer and adjusted to 2×10^6 spores/ml.

Each flask was inoculated with 1 ml spore suspension. The flasks were incubated for 7 days at 30 °C in a shaking incubator (160 rpm). The culture medium was then filtered through Whatman filter paper No. 1 to separate the mycelium from the culture filtrate. Replicates were made and the mycelial mat was oven dried at 80 °C till constant weight to obtain the dry weight expressed as mg ml^{-1} growth medium. The percentage of inhibition was counted as mentioned before.

3. Results and discussion

3.1. IR characterization of CMCh and CMCh-g-PNVI

FTIR spectra of both Chitosan and CMCh – Fig. 1 – show the strong peak at 1412 cm^{-1} which could be assigned to the symmetrical stretching vibration of COO^- group (Brugnerotto et al.,2001). The asymmetrical stretching vibrating of COO^- group near 1550 cm^{-1} is overlapped with the deforming vibration of NH_2 at 1600 cm^{-1} to obtain a very strong peak. The C–O absorption peak of the secondary hydroxyl group became stronger and moved to 1074 cm^{-1}. The results – which are in accordance with the work of (Xie et al., 2002a, 2002b) – indicated that the substitution occurred at C_6 position.

FTIR spectrum of the grafted CMCh (Fig 2) has proved that the initiation step for grafting was done on the amino group at C_2. This is well illustrated by the disappearance of the doublet band at 3445 and 3422 cm^{-1} corresponding to the $-NH_2$ group and the appearance of a singlet band at 3400 cm^{-1} for –NH group which indicates the abstraction of a H-atom by the $KSO_4^{\cdot}$ radical derived from the decomposition of the potassium persulphate initiator.

$$Ch{-}NH_2 + KSO_4^{\cdot} \rightarrow Ch{-}NH^{\cdot} + KHSO_4$$

The absorption band of the –OH of the –COOH group in CMCh is observed at 3000–2700 cm^{-1} and the –OH groups of the CMCh have also an absorption band at 3400–3200 cm^{-1}, so there is an overlap between many bands in this area screening the absorption band of –NH group. Whereas, in the IR chart of grafted copolymer (CMCh-g-PNVI), 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).

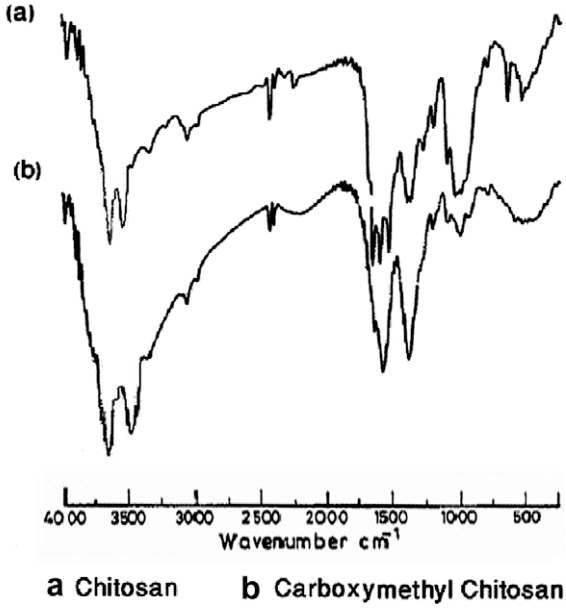


Fig. 1. Changes of IR peaks intensities of Chitosan and CMCh. (a) Chitosan (b) CMCh.

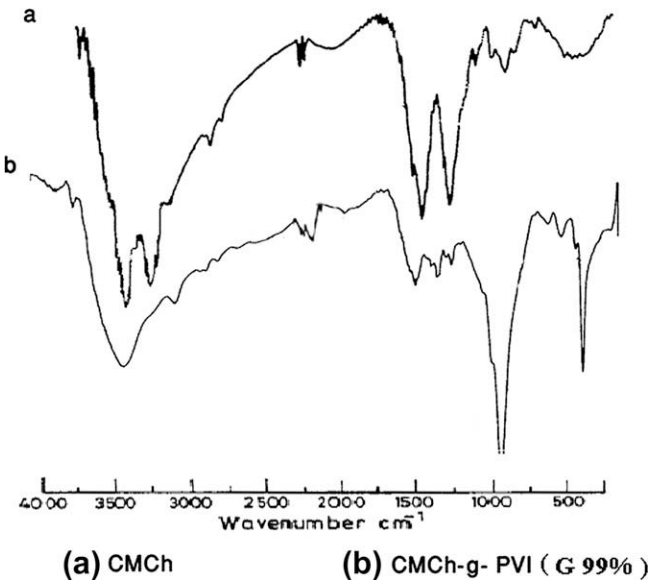


Fig. 2. Changes of IR peaks intensities of CMCh and CMCh-g-PNVI (G 99%).

Besides, C–H bond vibration of imidazole ring is observed at around 826 cm^{-1} – Fig 2. Therefore, it can be deduced that PNVI was incorporated onto CMCh backbone.

FTIR spectral study of grafted copolymers with various degree of grafting revealed that the intensities of C=C, C=N and C–H vibration bands of imidazole ring increase as the % grafting increases.

3.2. Thermogravimetric analyses of CMCh, PNVI and CMCh-g-PNVI

Thermogravimetric analyses of CMCh, its grafted copolymer with PNVI (G 99%) together with that of PNVI homopolymer are illustrated in Fig 3 and summarized in Table 1. The results clearly show the initial decomposition temperature (IDT) of PNVI homopolymer (150 °C) as compared with those of CMCh (258 °C) and CMCh-g-PNVI (G 99%) (220 °C). However, modification of CMCh by grafting with PNVI didn't improve its IDT value but increases its thermal stability by decreasing its rate of thermal degradation which was found to be slower for the grafted copolymer as compared with the parent CMCh.

It was found that PNVI loses 10% of its original weight at 185 °C and CMCh loses 10% of its weight at 160 °C, whereas the grafted copolymer (G 99%) loses 10% of its weight at a higher temperature (230 °C) than both CMCh and PNVI homopolymer. It was found that PNVI loses 30% of its initial weight at 345 °C and CMCh loses 30% of its weight at 280 °C, whereas the grafted copolymer loses 30% of its weight at a higher temperature (340 °C) than ungrafted CMCh.

Moreover, the weight loss at 500 °C was found to be lower for the grafted copolymer (41%) relative to the parent CMCh (55%) and PNVI (70%).

This improvement in the thermal stability of CMCh grafted samples is mainly attributed to the imidazole aromatic rings which are known to be thermally stable (Chi & Collier, 1988; Zhao, Sh, Wen, Peng, & Huang, 2006), which will lead to a delay in the degradation process.

3.3. Scanning electron microscopy: (magnification 1000×)

The scanning electron micrographs of Chitosan, CMCh, CMCh-g-PNVI (G 99%) and CMCh-g-PNVI (G 197%) are shown in Fig. 4. The

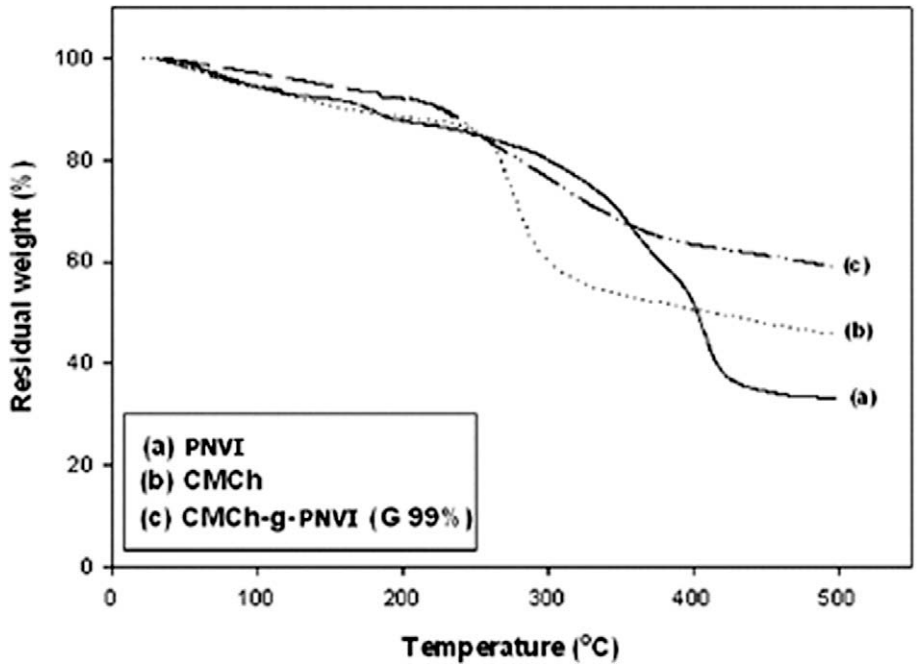


Fig. 3. Thermogravimetric curves of CMCh, CMCh-g-PNVI copolymer and PNVI homopolymer. (a) PNVI, (b) CMCh, (c) CMCh-g-PNVI (99%).

Table 1
Thermogravimetric analyses for PNVI homopolymer, CMCh and CMCh-g-PNVI copolymers (G 99%).

Type of polymer	IDT (°C)	Temp. at 10% wt. loss	Temp. at 20% wt. loss	Temp. at 30% wt. loss	Wt. loss % at 500 °C
PNVI	150	185	300	345	70
CMCh	258	160	260	280	55
CMCh-g-PNVI (99%)	220	230	280	340	41

flaky fibrous nature of chitosan was totally modified due to the formation of carboxymethyl chitosan (CMCh) as there appear more lumps on the smooth surface of chitosan due to the formation of –COOH groups. CMCh-g-PNVI showed a complete change in the morphology of the surface as it has more grooves on it which increase by increasing the percentage of grafting.

3.4. Solubility

Solubility results of the grafted products are summarized in Table 2. The prepared copolymers are found to be water soluble irrespective to the % grafting. The solubility of the grafted copolymers in water widens their applications for biological activity.

Also, the grafted copolymers were found to be freely dissolve in 1% acetic acid, but the solubility starts to decrease in acetic acid:ethanol mixture (1:1) then they become totally insoluble in ethanol, DMF, dioxane and THF solvents.

3.5. The antimicrobial activity of CMCh-g-PNVI copolymers

The antimicrobial activity of chitosan depends on several factors such as the kind of used chitosan (deacetylation degree, molecular weight), medium pH, incubation temperature, etc. Several hypotheses elucidating the antimicrobial activity of chitosan have been postulated. The most feasible hypothesis is a change in cell permeability due to interactions between the polycationic chitosan and the electronegative charges on the cell surfaces. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (Chen, Liau, & Tsai, 1998; Feng et al., 2000; Papineau, Hoover, Knorr, & Farkas, 1991; Sudarshan, Hoover, & Knorr, 1992; Young, Kohle, & Kaus, 1982).

Other mechanisms are the interaction of diffused hydrolysis products with microbial DNA, which leads to inhibition of mRNA and protein synthesis (Hadwiger, Kendra, Fristensky, & Wagoner, 1986) and the chelation of metals, spore elements and essential nutrients (Cuero, Osuji, & Washington, 1991).

Table 3 shows the effect of exposure of the Gram positive *S. aureus* and the Gram negative *E. coli* to different chitosan- and CMCh-g-PNVI. Chitosan and CMCh caused decreases in viable cell counts of *S. aureus* and *E. coli*. It was observed that while chitosan, compared with CMCh, was much effective in decreasing the viable cell count of *S. aureus* (51.7% and 25.0% inhibition for chitosan and CMCh, respectively), CMCh was more potent (68.9% inhibition) in case of *E. coli* than chitosan (41.4%). The same manner followed for PNVI grafts, where chitosan-g-PNVI (G 107%) caused higher inhibition (61.7%) in the viable cell count of *S. aureus* than CMCh-g-PNVI (G 99%) which caused 46.7% inhibition. Also, CMCh-g-PNVI (G 99%) had higher inhibitory activity on *E. coli* (93.1%) than chitosan-g-PNVI (G 107%) with 74.1% inhibition. It was obvious that increasing the graft percentage in each case caused higher inhibition of viable cell counts of both *S. aureus* and *E. coli*. Generally, the antibacterial activities of the tested polymers were stronger against *E. coli* than *S. aureus*. This might be attributed to their different cell walls. *E. coli* is a typical Gram negative bacterium, the cell wall of which is made up of a thin membrane of peptidoglycan and an outer membrane constituted of lipopolysaccharide, lipoprotein and phospholipids. Chitosan and its derivatives with large molecular weight can be expected to coat the cell surface and prevent the leakage of intracellular components (Zhong et al., 2008). Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001 have shown that chitosan disrupted the barrier properties of the outer membrane of Gram negative bacteria, as chitosan is protonated at acidic conditions and the carboxyl and phosphate groups of the bacterial surface are anionic and offer potential sites for elec-

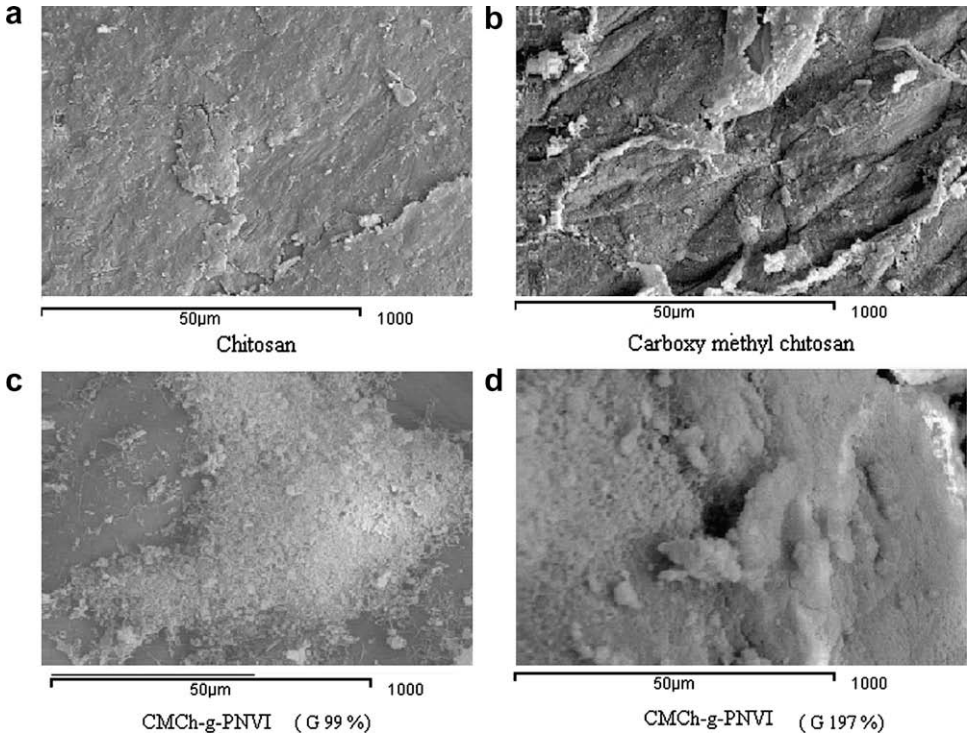


Fig. 4. Scanning electron micrographs of Chitosan, CMCh, CMCh-g-PNVI (G 99%) and CMCh-g-PNVI (G 197%).

Table 2
Solubility of CMCh-g-PNVI (G 99%) and (G 197%).

Solvent	Solubility of CMCh-g-PNVI copolymers	
	G 197%	G 99%
Distilled water	Soluble	Soluble
1% acetic acid	Soluble	Soluble
1% acetic acid:ethanol (1:1)	Cloudiness	Cloudiness
Ethanol	Insoluble	Insoluble
DMF	Insoluble	Insoluble
1,4-Dioxane	Insoluble	Insoluble
THF	Insoluble	Insoluble

Table 3
Effect of chitosan- and CMC-PVI derivatives on viable cell count (CFU ml^{-1}) of *S. aureus* and *E. coli*.

Sample	*S. aureus*		*E. coli*	
	CFU ml^{-1} ($\times 10^7$)	Inhibition (%)	CFU ml^{-1} ($\times 10^7$)	Inhibition (%)
Untreated	6.0	–	5.8	–
Chitosan	2.9	51.7	3.4	41.4
CMCh	4.5	25.0	1.8	68.9
Chitosan-g-PNVI (G 107%)	2.3	61.7	1.5	74.1
Chitosan-g-PNVI (G 192%)	2.1	65.0	0.7	87.9
CMCh-g-PNVI (G 99%)	3.2	46.7	0.4	93.1
CMCh-g-PNVI (G 197%)	2.8	53.3	0.14	97.6

Data represented are means of three replicates.

trostatic binding. The NH group in the poly (vinyl imidazole) derivatives can be protonated under acidic conditions, so it can react with the carboxyl and phosphate groups of the bacterial surface and therefore show antibacterial activity against Gram negative bacteria. On the other hand, *S. aureus* a typical Gram positive bacterium has cell wall composed mainly of peptidoglycan, which does not allow the formation of a surface layer.

Data in Table 4 reveal the antifungal activities of the Chitosan-g-PNVI and CMCh-g-PNVI derivatives when tried on the plant pathogenic fungus *F. oxysporum* and the human pathogenic fungus A. fumigatus. Chitosan showed an inhibitory effect on *F. oxysporum* growth (6.8%). On the contrary, the dry weight of *A. fumigatus* increased on applying chitosan to the growth medium denoting a degrading ability towards the used Chitosan. It was reported that *A. fumigatus* has a strong degradation ability for some polymers (Eldsater, Erlandsson, Renstad, Albertsson, & Karlsson, 2000; Osawa, Yabe, Miyamura, & Mizuno, 2006). Clearly, Grafting of vinyl imidazole onto Chitosan and CMCh was successful in inhibiting fungal growth. The 99% PNVI graft on CMCh exhibited a higher effect (48.6% and 38.5% inhibition for *F. oxysporum* and *A. fumigatus*, respectively) compared with the 107% PNVI graft on chitosan (20.6% and 15.0% inhibition for *F. oxysporum* and *A. fumigatus*, respectively). Increasing the graft percentage led to higher suppression of growth. It was found that the CMCh-g-PNVI (G 197%)) caused the highest inhibition (63.3% and 54.0% for *F. oxysporum* and *A. fumigatus*, respectively). In each case, the plant pathogen *F. oxysporum* was more vulnerable towards the tested polymers than *A. fumigatus*.

Table 4
Effect of chitosan- and CMC-PVI derivatives on dry mycelial weight (mg ml^{-1}) of *F. oxysporum* and *A. fumigatus*.

Sample	*F. oxysporum*		*A. fumigatus*	
	Dry wt. (mg ml^{-1})	Inhibition (%)	Dry wt. (mg ml^{-1})	Inhibition (%)
Untreated	4.4	–	4.5	–
Chitosan	4.1	6.8	5.1	−11.9
CMCh	3.8	12.4	4.1	10.2
Chitosan-g-PNVI (G 107%)	3.5	20.6	3.8	15.0
Chitosan-g-PNVI (G 192%)	3.1	28.9	3.5	23.0
CMCh-g-PNVI (G 99%)	2.2	48.6	2.8	38.5
CMCh-g-PNVI (G 197%)	1.6	63.3	2.1	54.0

Data represented are means of three replicates.

Imidazole drugs have well defined antifungal range (Strippoli, D'Auria, Tecca, Callari, & Simonetti, 2000; Tullio, Cuffini, & Carlone, 1990; Zampieri et al., 2008). A number of biochemical targets have been proposed for the mechanism of azole activity including impairment of the barrier function of the membrane and the inhibition of the cytochrome P-450 mediated sterol demethylase (Bossche, 1974; Kerridge, 1986).

3.6. Kinetic studies of grafting

The effect of the initiator concentration on the extent of grafting is represented in Fig. 5. The initiator concentration was varied from 3 to 9×10^{-2} mol/L keeping the monomer concentration constant at 1 mol/L, the temperature at 60 °C, the reaction time at 2.5 h. Both the %G and %GE increase with increasing the initiator concentration reaching its maximum value at 8×10^{-2} mol/L. A further increase in KPS 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 through chain-transfer to initiator or due to the coupling between initiator radicals (Hamit et al., 2007).

The effect of NVI concentration on the grafting process is illustrated in Fig. 6. The monomer concentration was studied within the range (0.5–3 mol/L), while the initiator concentration was kept constant at 8×10^{-2} mol/L, the temperature at 60 °C, the reaction time at 2.5 h. The data revealed the increase in both the graft yield (%G) and graft efficiency (%GE) reaching its maximum value at 1 mol/L monomer concentration, then starts 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 proposed (Bamford & Schofield, 1981) in

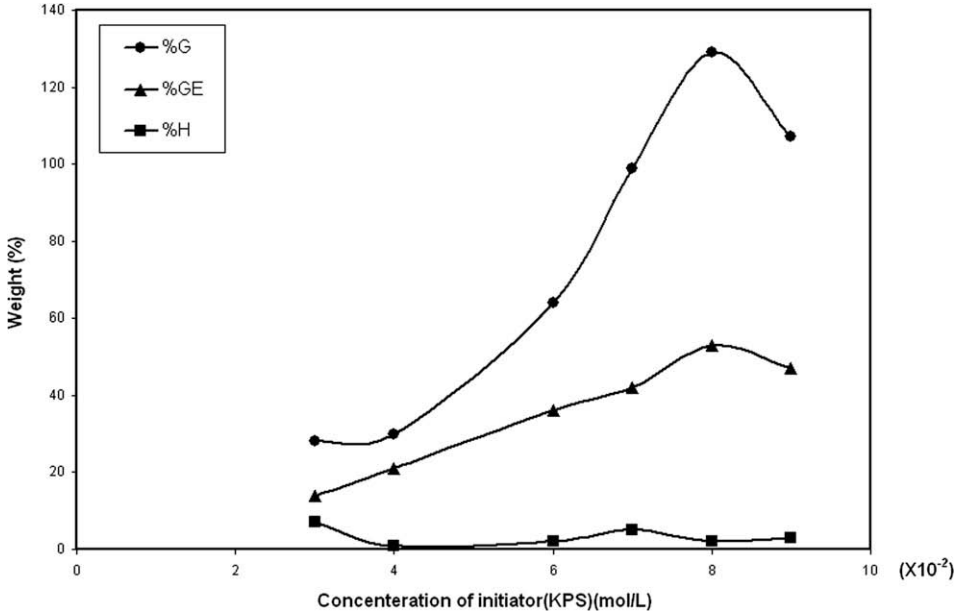


Fig. 5. Effect of initiator concentration (KPS) on percentage grafting (%G), grafting efficiency (%GE) and percentage homopolymer (%H), time = 2 h, temp. = 60 °C, [M] = 1.5 mol/L.

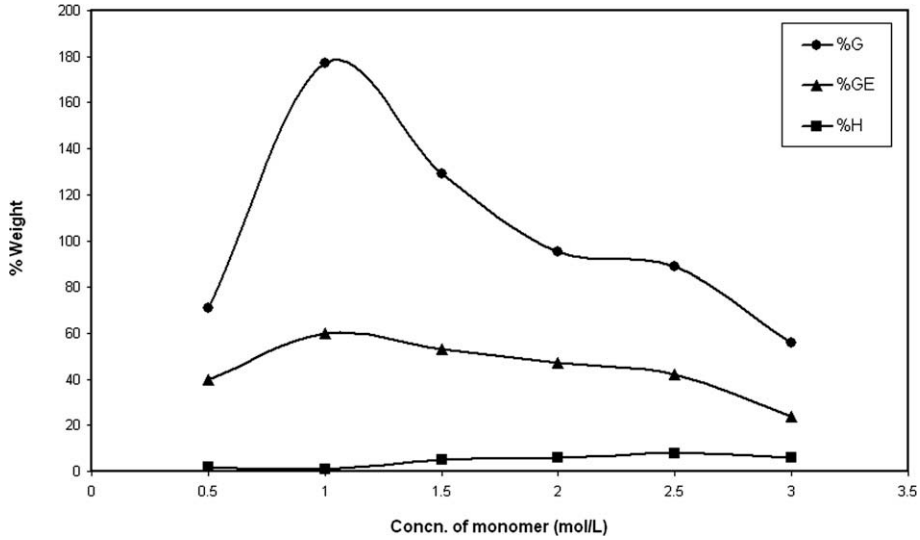
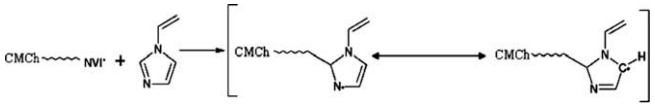


Fig. 6. Effect of monomer concentration (NVI) on percentage grafting (%G), grafting efficiency (%GE) and percentage homopolymer (%H), time = 2 h, temp.=60 °C, [KPS] = 8×10^{-2} mol/L.

Scheme 1, which 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 carboxymethyl chitosan backbone, which creates steric hindrance for further grafting.

The effect of reaction temperature on percentage of grafting as well as grafting efficiency and homopolymer formation is shown in Fig. 7. The reaction temperature varies from 55 to 70 °C keeping the initiator concentration constant at 8×10^{-2} mol/L, the monomer concentration at 1 mol/L, the reaction time at 2.5 h. It is apparent that the optimum temperature for maximum grafting is 60 °C.



Scheme 1.

Further increase in temperature reduces the percentage of grafting. A similar trend was observed for percent grafting efficiency (Hamit et al., 2007). At higher temperatures chain-transfer reactions are favored decreasing the extent of grafting.

The influence of reaction time on grafting is shown in Fig. 8. Increasing the reaction time (from 1 to 4 h) produces an increase in grafting while keeping the reaction temperature constant at 60 °C, the initiator concentration at 8×10^{-2} mol/L and the monomer concentration at 1 mol/L, At longer reaction times, the percentage of grafting begins to level off. The percentage of grafting reaches a plateau after 2.5 h due to the depletion of the monomer, initiator and available grafting sites as the reaction proceeds. Grafting efficiency also shows a similar trend.

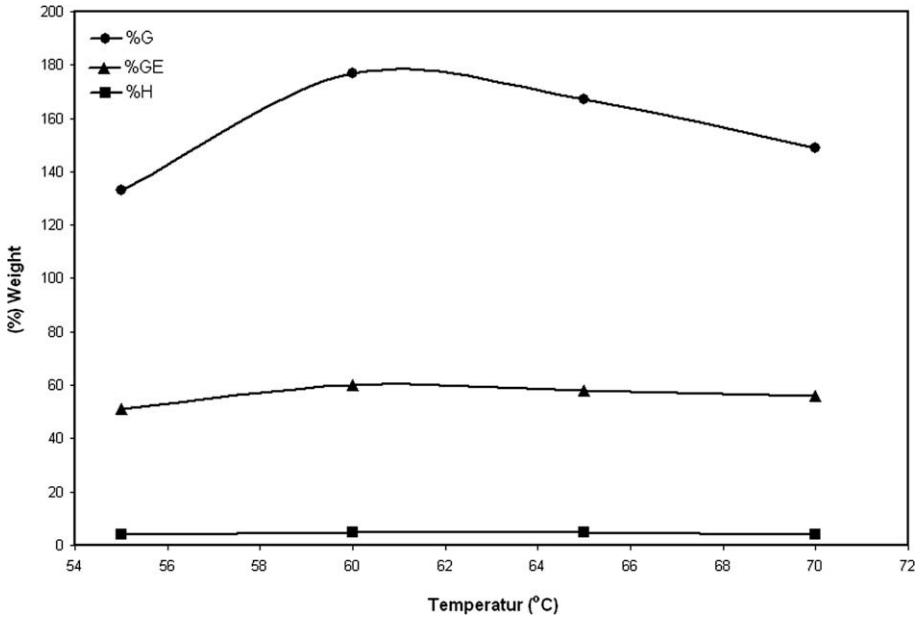


Fig. 7. Effect of reaction temperature on percentage grafting (%G), grafting efficiency (%GE) and percentage homopolymer (%H), time = 2 h, [KPS] = 8×10^{-2} mol/L, [M] = 1 mol/L.

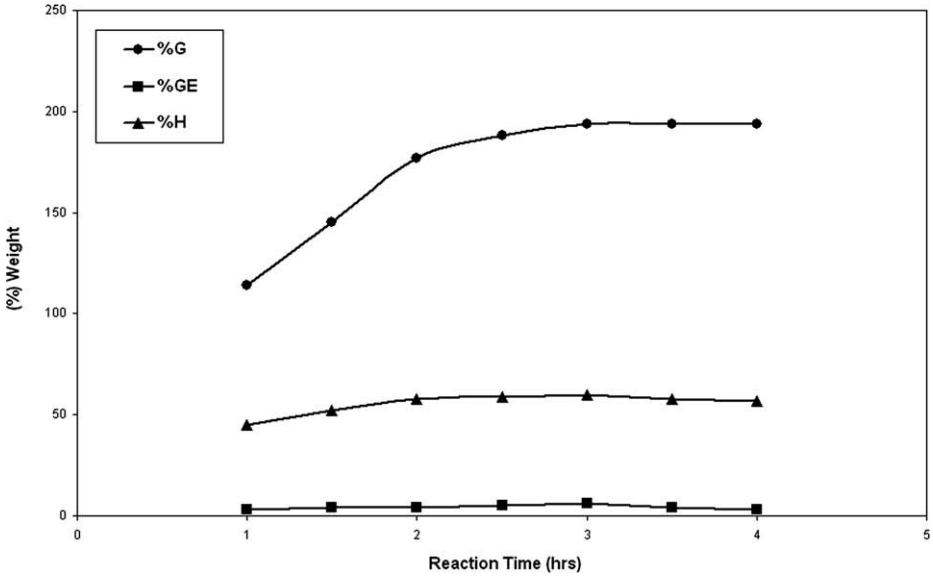


Fig. 8. Effect of reaction time on percentage grafting (%G), grafting efficiency (%GE) and percentage homopolymer (%H), temp = 60 °C, [KPS] = 8×10^{-2} mol/L, [M] = 1 mol/L.

However, the lowering in the %H as a function of reaction time may be attributed to the possible coupling of the macro radicals formed in the medium with the macro radicals grafted onto CMCh especially at the later stages of polymerization. These results were found to be in accordance with the work done by (Hamit et al., 2007).

4. Conclusions

1. In the present work, carboxymethyl chitosan was prepared, characterized by FTIR, SEM and TGA, then it was modified by graft copolymerization of *N*-vinyl imidazole onto its backbone.
2. TGA studies showed that the thermal stability of the copolymers CMCh-g-PNVI were found to be better than CMCh and the thermal stability increases with the increase in graft %. Moreover, it was obvious from SEM results that carboxymethylation of chitosan and its graft copolymerization by PNVI modified the flaky nature of its surface morphology.
3. Grafting was confirmed by various tools and the effects of different reaction parameters onto the grafting yield were investigated. Optimum grafting conditions were reported as follows: [I] = 8×10^{-2} mol/L, [M] = 1 mol/L, reaction temperature = 60 °C and reaction time = 2.5 h.
4. Chitosan, CMCh and their grafted copolymers caused decrease in viable cell counts of *S. aureus* and *E. coli*. The antibacterial activities of these derivatives against *E. coli* are stronger than against *S. aureus*. Grafting of vinyl imidazole onto Chitosan and CMCh was successful in inhibiting fungal growth. The increase in grafting percentage leads to higher suppression of fungal growth.

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