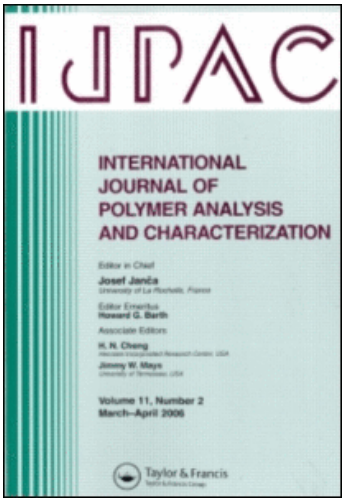

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Preparation and Characterization of Maleic Acid Grafted Chitosan

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Abstract: Modification of chitosan (CHI) with maleic acid (MA) via maleic anhydride (MAn) was investigated in acetic acid solution, in the presence of cerium ammonium nitrate (CAN) as the initiator. The effects of reaction time, temperature, and CAN and MA concentrations on the grafting percentage were studied. The products were characterized by Fourier-transform infrared, differential scanning calorimetry, and ^{13}C NMR, in addition to gravimetric evidence to grafting. A maximum of 105% grafting was achieved under the reaction conditions of 0.12 M CHI, 2.0 M MA, and 0.091 M CAN by stirring for 3 h at 70°C. Dissolution and swelling properties of grafted products were tested in the pH range 3–11. Products up to 36.5% grafting were found to be soluble in aqueous solution, while those with higher grafting percentages (48–81%) were partly soluble in the pH range studied. The product with maximum grafting percentage (105%) swelled and acted as a polyampholyte gel.

Keywords: Chitosan; Maleic anhydride; Cerium ammonium nitrate; Water soluble; Stimulus responsive

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INTRODUCTION

Chitin, poly[β-(1→4)-2-acetamido-2-deoxy-D-glucose], which is found in the outer shells of crustaceans, is the second most abundant polysachharide after cellulose. This natural polymer, having structural and chemical resemblance to cellulose, offers a broader range of applications than cellulose due to the presence of the acetamido group on the second carbon of the pyranose ring. Chitosan, poly[β-(1→4)-2-amino-2-deoxy-D-glucose], is the *N*-deacetylated form of chitin and has many useful features such as hydrophilicity, biocompatibility, biodegradability, antibacterial property, ion-chelating ability, and a remarkable affinity for many proteins and fats.[1] Chitosan is easier to work with than chitin due to the protonation of amine groups in dilute acid solutions, especially acetic acid, leading to solubility. Substantial efforts have been made by many researchers on chemical modification of chitosan aiming to improve the solubility and reactivity of the polymer and hence diversify its applications media.

Grafting of vinyl monomers onto chitosan is one way of achieving chemical modification of this aminopolysaccharide. Several studies concerning graft copolymerization on chitin or chitosan have been reported. Redox initiators like Fenton's reagent, potassium persulfate, and cerium (IV) ammonium nitrate (CAN) have been found to be effective for this purpose.[2–4]

Acylation, aldimination, carboxymethylation, sulfation, butyrilation, and formylation of chitin or chitosan have been achieved through classical methods of organic synthesis.[5–7] Sashiwa and Shigemasa[8] prepared *N*-acylated chitosan derivatives soluble at various pH values via ring opening reactions with various cyclic anhydrides in aqueous methanol system. Although these chitosan derivatives were soluble in both acidic and basic media, they were all insoluble at pH values 5–7.

Although maleic acid is not an ordinary vinyl monomer, it has been reported to polymerize in water in the presence of potassium persulfate and poly(*N*-vinyl pyrrolidinone).[9] Therefore, it seemed worthwhile to investigate the possibility of grafting this monomer onto chitosan through redox initiation by CAN, at ambient temperatures. Polyampholytes based on chitosan and maleic acid were prepared with varying degrees of grafting. The relationship of solubility behavior in aqueous solution with grafting percentage was investigated. The pH-sensitive swelling behavior and the swelling mechanism of the insoluble product are reported here. While soluble products could be useful as antimicrobial agents, the insoluble ones could find pharmaceutical applications, since they exhibit gel-like properties.

EXPERIMENTAL SECTION

Materials

Chitin powder (Sigma Chemicals Co.) was treated with 0.1 M HCl and 0.1 M NaOH solutions and washed several times with acetone. Chitosan (CHI) produced from the powdered chitin was used without further purification. Ceric ammonium nitrate (Aldrich), maleic anhydride (MAn) (Sigma), acetic acid (Merck), and acetone (Merck) were used as received.

Preparation of Chitosan from Chitin

Chitin was deacetylated to chitosan by treating it with 50% w/v NaOH solution under reflux at 109°C for 3 h. Chitosan samples obtained were washed with distilled water until neutral and then dried in oven at 60°C.

Grafting

Chitosan (0.2 g) dissolved in acetic acid solution (10 mL, 1% v/v) was purged with nitrogen gas, followed by adding MAn, which hydrolyzes readily to maleic acid (MA) in aqueous solutions. Then CAN as a redox initiator was added into the reaction medium. The mixture was left for 3 h at 70°C in nitrogen atmosphere under continuous stirring. The reaction was terminated by adding water. Acetone was used to precipitate the product. Any unreacted monomer, MA, was removed by extraction with acetone. The product was then dried at 60°C.

Reaction variables studied were: monomer concentration (0.095, 0.13, 0.16, 0.22, 0.25, 0.31, 1.0, 2.0, 3.0, and 5.0 M), initiator concentration (0.00, 0.018, 0.091, 0.13, and 0.18 M), temperature (60°, 70°, and 80°C), and time (30, 60, 120, 180, and 300 min).

Percent grafting was calculated by the following equation:

$$Grafting(\%) = \frac{copolymer(g) - chitosan(g)}{chitosan(g)} \times 100\% \qquad (1)$$

Characterization of the Products

Degree of Deacetylation of Chitosan

The degree of deacetylation of chitosan was determined by the titrimetric method.[10] Chitosan-HBr salt was titrated against 0.1 M standardized

NaOH solution and the degree of deacetylation of chitosan was found to be 60%.

Molar Mass of Chitosan

Chitosan molecular weight was determined by dilute solution viscometry at 30°C in a 0.1 M acetic acid/0.2 M sodium acetate buffer solution. The equation given below[11] was used to calculate the viscosity-average molecular weight of the sample prepared:

$$[\eta] = 1.04 \times 10^{-4} M^{1.12} (\mathrm{mL/g}) \quad (2)$$

M_v was calculated to be 1.24×10^6 g/mol.

^{13}C NMR

The samples were studied in the solid state by ^{13}C cross-polarization/magnetic angle spinning (CP/MAS) at 8 kHz spinning rate; 3 s repetition time; 1 ms contact time and 15000 scans. Line integrals were normalized at C-1 signal of chitosan at 105 ppm.

FTIR

Samples were analyzed by Fourier transform infrared (FTIR) spectroscopy in the solid state using KBr pellets with a Mattson Satellite 5000 FTIR Spectrometer, equipped with Winfirst software.

Differential Scanning Calorimetry

DSC was carried out using a General V4.1 DuPont 2000 calorimeter. The samples were tested under N_2 atmosphere. The heating rate was 10°C/min.

Solubility and Swelling Studies

The solubility and swelling properties of the products were studied in different buffer solutions of the pH range 3 to 11 at room temperature. Buffer solutions used were: acetate buffer (pH 3 and pH 5), citrophosphate buffer (pH 7), and boric buffer (pH 9). Sodium hydroxide solution was used to obtain a pH value of 11. The swelling behavior of the insoluble product was studied in the above-mentioned buffer solutions. The weight of completely dried samples was measured, and the samples were dipped into tubes filled with a different pH buffer solution at

25°C. The weight of the swollen samples was measured within the time intervals of 10, 20, 1440 (24 h), and 2880 (48 h) min.

The swelling characteristics of the products are determined in terms of equilibrium swelling ratio (Q_e):

$$Q_e = \frac{w_e}{w_0} \tag{3}$$

where w_0 is the initial weight of the dry sample and w_e is the weight of swollen sample at equilibrium.

RESULTS AND DISCUSSION

Grafting yield depends upon a large number of variables such as initiation method, nature of the monomer, type of the substrate, reaction medium, reaction temperature, and reaction time. In the present study, grafting of chitosan was studied with respect to MA and CAN concentration, temperature, and time. It has been proposed that grafting of a monomer onto a cellulose type of substrate by redox initiation involves a complex formation between the OH groups of the polymer, the transition metal ion, and the monomer.[12] In the case of chitosan and Ce^{4+} ion, the neighboring $-OH$ and $-NH_2$ groups on the second and third carbons of the pyranose ring are involved in a complex formation with Ce^{+4} ion resulting in the formation of free-radical centers on the chitosan backbone.

Effect of MA and CAN Concentrations on Grafting Percentage

Since grafting is due to the formation of a complex between the substrate, monomer, and Ce^{4+} ion, the efficiency of grafting will be highly dependent on the monomer and initiator concentrations.

The effect of MA concentration on percent grafting at [CAN] = 0.091 M and [CHI] = 0.12 M at 70°C was investigated in the concentration range from 0.095 to 5.0 M as shown in Figure 1. Within a MA concentration of 0.095–0.16 M, average grafting was 19%. This value increased to 33% in the concentration range 0.22–0.31 M and to 64% when the concentration of MA was increased to 1.0 M. At 2.0 M concentration, a maximum value of 105% was obtained. However, a further increase in MA concentration (3.0 and 5.0 M) resulted in lower grafting percentages.

Incorporation of MA onto chitosan, however, could not be achieved in the absence of CAN with 0.31 M MA and 0.12 M chitosan in 10 mL

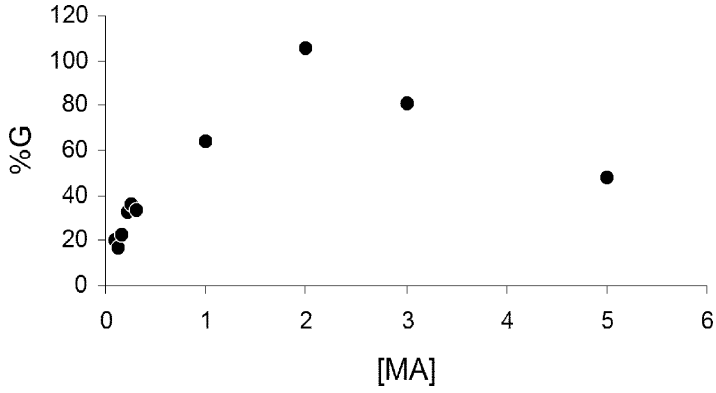

Figure 1. Effect of [MA] on % grafting.

acetic acid solution under nitrogen atmosphere. Increasing CAN concentration from 0.018 to 0.13 M resulted in an increase in the grafting yield from 21.5 to 59.0%. When [CAN] is increased to 0.18 M, grafting decreases to 42%, as shown in Figure 2.

Although, in general, the extent of grafting increases with increase in monomer or initiator concentration, there is always a limitation beyond

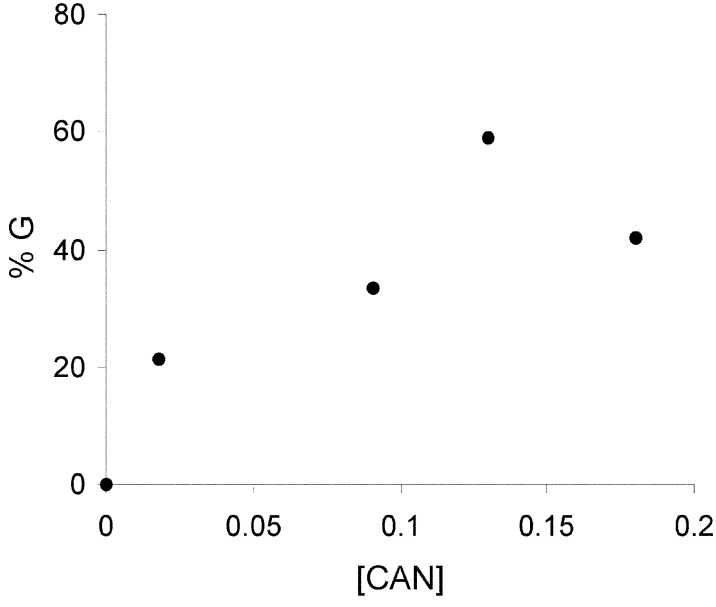

Figure 2. Effect of [CAN] on % grafting.

Table I. Initial pH values of CHI/CAN/MA systems studied

Sample	[MA]	[CAN]	Initial pH
6	0.31	0.091	1.3
8	2.0	0.091	1.3
10	5.0	0.091	0.8
11	0.31	0.13	1.0
12	0.31	0.18	0.9

0.2 g (0.12 M) chitosan, 10 mL HAc.

which grafting is not favored. In the CHI/MA/CAN system, MA or CAN concentrations higher than 2.0 or 0.13 M respectively cause an increase in the acidity of the medium. At constant CAN concentration of 0.091 M, initial pH of the grafting system decreases from 1.3 to 0.8 when MA concentration is increased from 2.0 to 5.0 M, as shown in Table I. Similarly, at constant MA concentration of 0.31 M, increasing CAN concentration of the system causes a decrease in pH from 1.3 to 0.9. A more acidic medium causes a decrease in grafting percentage, which may be attributed to an abundance of protons acting as radical terminators.

Effect of Temperature and Time on Grafting Percentage

A significant effect of neither temperature nor time could be observed on percent grafting. An average of 33% grafting was obtained for a MA/CAN/CHI 0.3/0.5/0.2 system within temperature and time intervals of 60–80°C and 30–300 min, respectively. NMR and FTIR analyses of the products obtained at temperatures lower than 60°C and reaction times less than 30 min did not reveal any indication of grafting of MA on the polysaccharide backbone. It seems that an induction period of 30 min and an induction temperature of 60°C satisfy the conditions for the occurrence of the cited reaction in solution. Although time and temperature are effective parameters in heterogeneous grafting reactions,[12–14] no such observation could be made in this study, which was carried out under homogeneous conditions.

^{13}C CP/MAS

Figure 3(a) shows the ^{13}C CP/MAS spectrum of chitosan. Carbonyl carbon and methyl carbons of *N*-acetyl group appeared at 174 and 24 ppm, respectively. The peaks at 106, 84, 76, 60, and 56 ppm belong

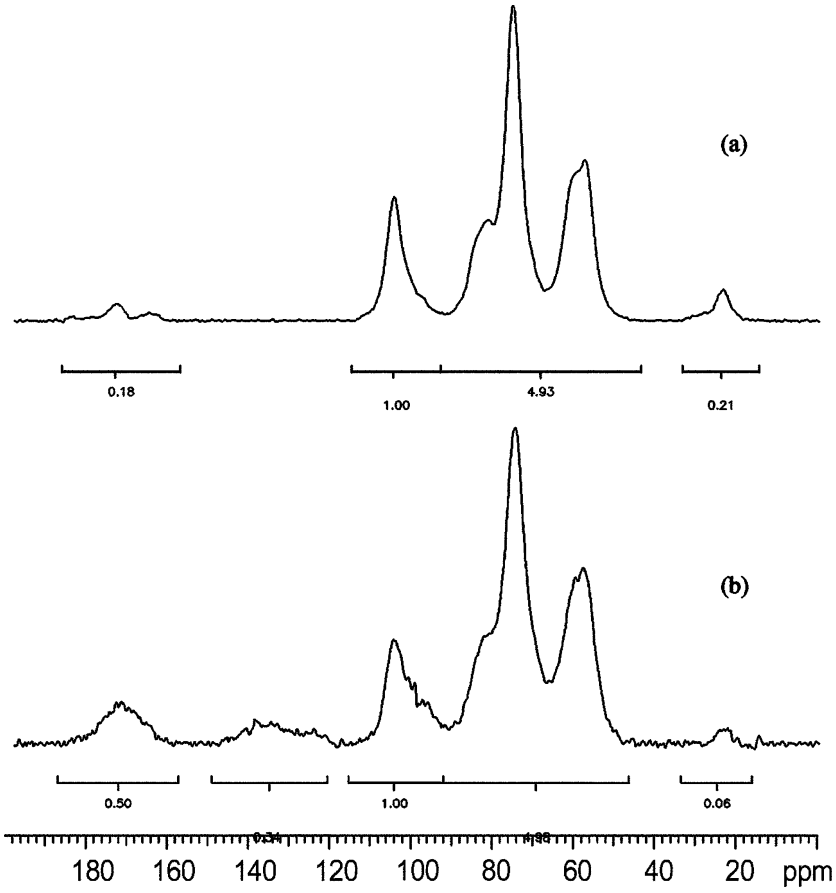

Figure 3. ^{13}C NMR spectra of (a) chitosan and (b) grafted chitosan.

to C-1, C-4, C-5, C-6, and C-2 carbons of the pyranose ring of chitosan, respectively.[15]

Figure 3(b) refers to Sample 6, the product obtained when chitosan dissolved in 1% acetic acid is treated with 0.31 M MA in the presence of 0.18 M CAN for 3 h. The three-fold increase in the area of the peak at 174 ppm belonging to the carbonyl groups and an additional broad peak at 136 ppm belonging to $-C=C-$ shows incorporation of maleic acid onto chitosan. The degree of grafting can be taken to be around 34% according to the peak area at 136 ppm. This result is in very good agreement with the gravimetric result of 33.5% for the same sample. The decrease in the area of the peak at 24 ppm (methyl of *N*-acetyl group) from 0.13 to 0.006 indicates some grafting on the amide nitrogen of chitosan.

FTIR

In Figure 4, FTIR spectra of chitosan and grafted chitosan are compared. Characteristic peaks of chitosan at around 3500 cm^{-1} are due to alcohol and amine groups, at 1655 and 1593 cm^{-1} are due to amide I

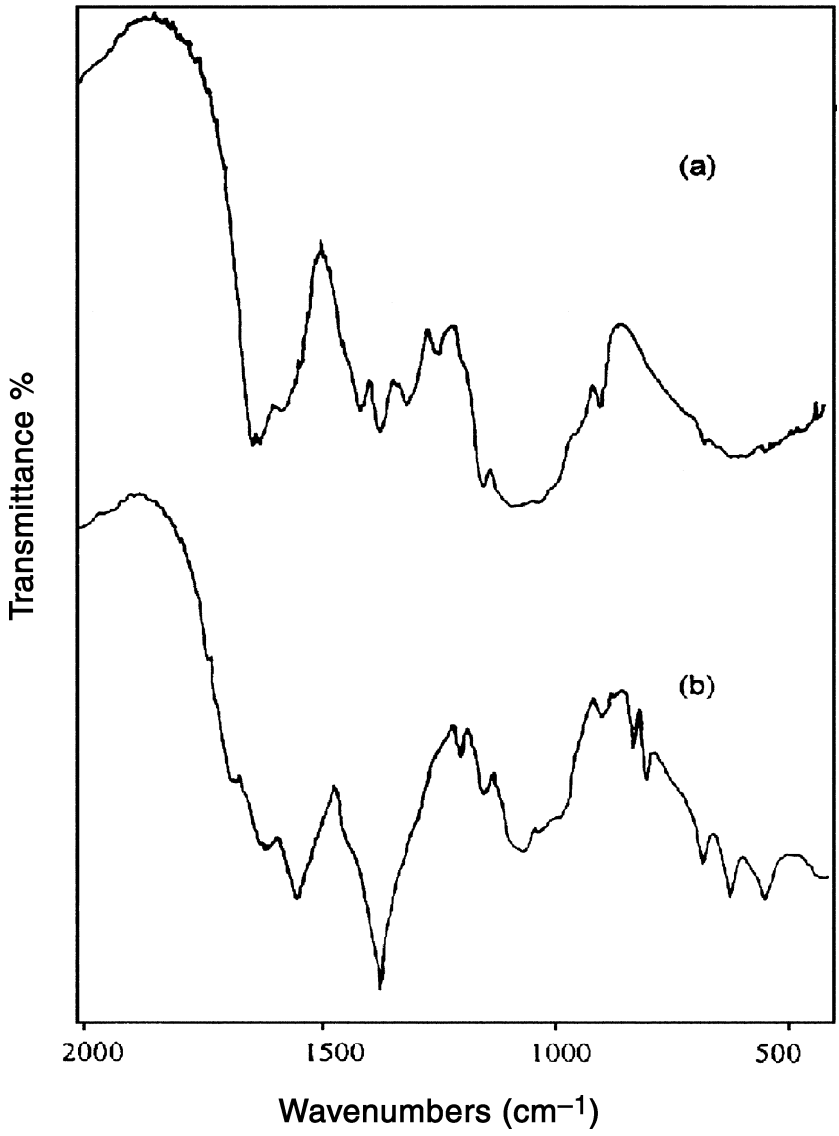

Figure 4. FTIR spectra of (a) chitosan and (b) grafted chitosan.

and amide II stretching, at 1375 cm^{-1} are due to methyl group of acetamido function, and at 1060 cm^{-1} peaks due to ether linkage in pyranose units are clearly observable. Amide I and amide II bands of chitosan are lost in the spectrum of grafted chitosan, as shown in Figure 4, line (b). New amide bands are observed at 1640 and 1570 cm^{-1}. O–H and C–H bending and C–N stretching overlap in the range 1400–1200 cm^{-1} with a sharp peak at 1383 cm^{-1}. The stretching bands of etheric linkages of the pyranose ring of chitosan appear unchanged in the product spectrum. The peaks at 827 cm^{-1} and 796 cm^{-1} are additional evidence for the incorporation of the maleic acid onto the chitosan backbone. These peaks are attributed to C–H rockings of olefinic structure. Also at 1714 cm^{-1}, a small new peak is identified, which is due to the C=O vibrations of maleic acid introduced onto the chitosan backbone.

DSC

DSC thermograms of chitosan and grafted chitosan are shown in Figure 5, lines (a) and (b), respectively. Chitosan decomposes with a characteristic sharp exothermic decomposition peak at 308°C. The grafted product exhibits two broad decomposition peaks at 175° and 250°C. The change in decomposition behavior is taken as an indication of the grafting of chitosan. The disruption of intermolecular H-bonding among chitosan chains is reflected in a lower decomposition temperature.

Dissolution Behavior

Solubility behavior of the grafted products, which is different than that of chitosan, is further evidence for grafting. The results of solubility tests for samples 1 to 10 in aqueous solution are summarized in Table II. Products with grafting lower than 48% are soluble in both acidic and basic media. A grafting percentage higher than 48% results in partly soluble or insoluble products. This behavior might be attributed to difunctionality of maleic acid monomer. Being difunctional, once grafted, it acts as a cross-linker between chitosan chains. Such products are either partly soluble or insoluble depending upon the degree of cross-linking. As can be followed from Table I, the critical MA concentration that initiates cross-linking is 2.0 M. The product with 105% grafting value is insoluble in aqueous solution but swells, exhibiting gel-like properties. Hence, products with desired dissolution properties could be obtained by controlling the degree of grafting.

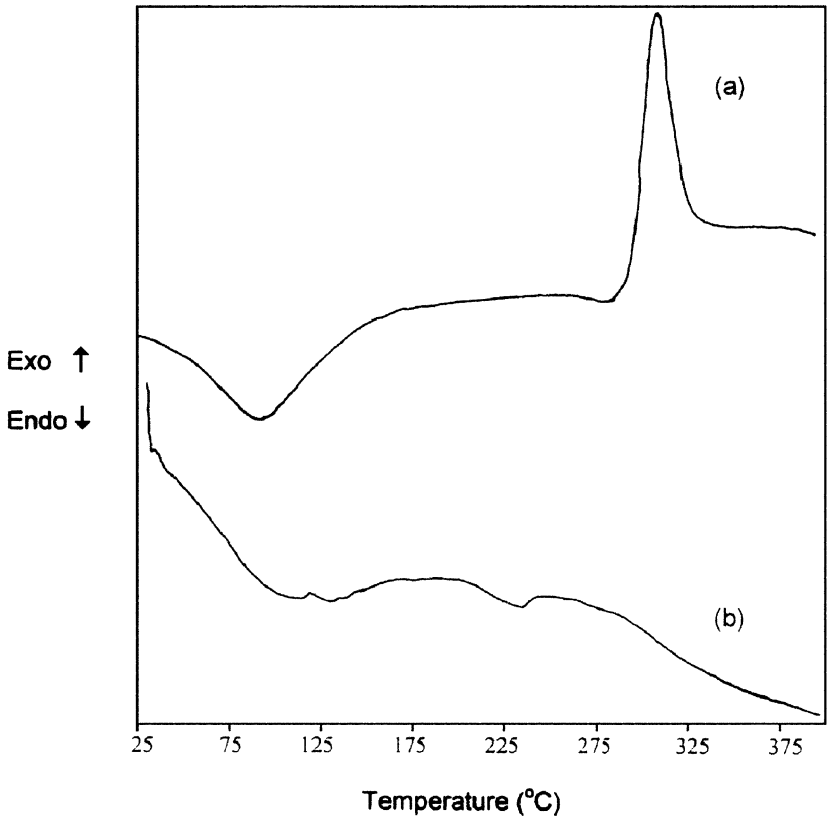

Figure 5. DSC thermogram of (a) chitosan and (b) grafted chitosan.

pH-Sensitive Swelling Behavior of Maleic Acid Grafted Chitosan

The cross-linked product (sample 8) with grafting of 105% was swollen in buffer solutions of pH 3, 5, 7, 9, and 11. The swelling behavior of the insoluble product was studied in the above-mentioned buffer solutions. The ratio of the weight of swollen sample at time t to the initial weight, namely swelling ratio (Q_t), was evaluated as w_t/w_0, where w_t is the weight of swollen sample at time t and w_0 is the initial weight of the sample. Figure 6 shows swelling behavior of the sample in aqueous solutions of various pH values.

Equilibrium is reached within 24 h in all solutions studied. Equilibrium swelling ratios, Q_e, are shown as a function of pH in Figure 7. Q_e ranging between 4 and 6 were obtained, indicating that maleic acid grafted chitosan is has excellent water absorbency. Moreover, swelling

Table II. Solubility of maleic acid modified chitosan samples in aqueous solutions

Sample	[MA]	% Grafting	Solubility at pH[a]				
			3	5	7	9	11
2	0.13	16.5	+	+	+	+	+
1	0.095	20.0	+	+	+	+	+
3	0.16	22.5	+	+	+	+	+
4	0.22	33.0	+	+	+	+	+
6	0.31	33.5	+	+	+	+	+
5	0.25	36.5	+	+	+	+	+
10	5.0	48.0	+	+	+	±	±
7	1.0	64.0	±	±	±	±	±
9	3.0	81.0	±	±	±	±	o
8	2.0	105.0	o	o	o	o	o

[a] + = soluble, ± = partly soluble, o = swollen.

behavior of the product is pH sensitive. A maximum swelling ratio of 6.4 was obtained at pH 3. A minimum Q_e value of 1.9 was obtained at neutral pH. The fact that the product is capable of swelling in both acidic and basic solutions shows that it is a polyampholyte gel. Depending on the ratio and degree of ionization of the amine and carboxylic acid groups present in the structure, the network swells. While repulsive forces

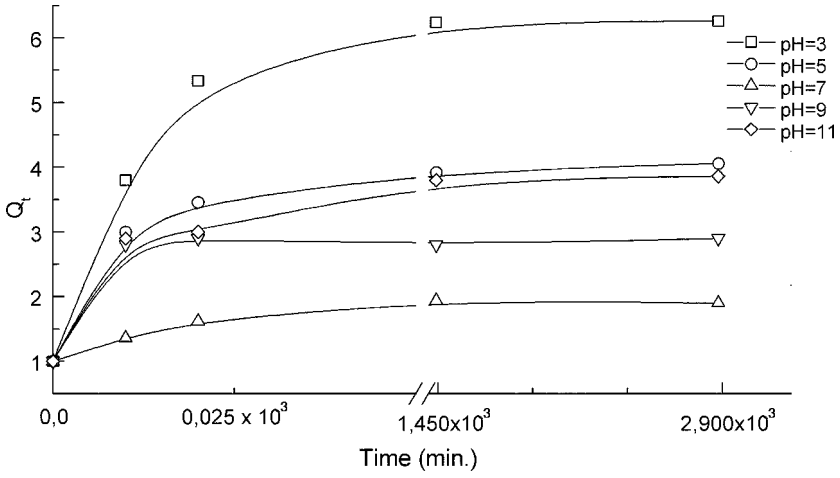

Figure 6. Swelling behavior of maleic acid grafted chitosan (sample 8).

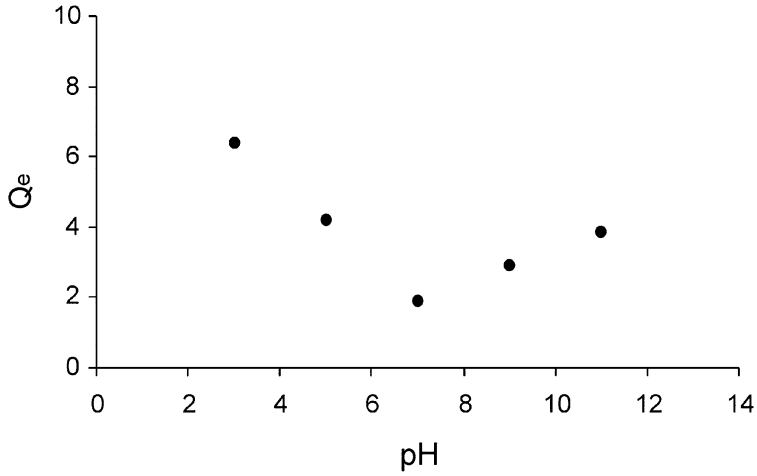

Figure 7. Equilibrium swelling ratio, Q, as a function of pH.

dominate at acidic and basic conditions, attractive forces are predominant at neutral pH, causing a minimum swelling to occur.

The swelling mechanism proposed was followed by FTIR analysis. Figure 8, lines (a), (b), and (c) show the FTIR spectra of the gel after being swollen at pH values 3, 7, and 11, respectively. The C–O stretching of carboxylic acid peak at 1714 cm^{-1} and N–H bending vibrations of the protonated amine ($-NH_3^+$) group at around 1560 cm^{-1} are observable at pH 3. The carboxylic acid peak is absent in the spectra of samples swollen at pH 7 and 11. Instead, bands at 1662, 1552, and 1530 cm^{-1} characterizing the carboxylate ion group and amine group at 1590 cm^{-1} are present.

Antibacterial Activity

Preliminary work done on the antibacterial activity of these soluble products showed enhanced antibacterial activity, especially on gram negative bacteria (*E. coli* and *P. aeruginosa*), when compared to chitosan alone. A detailed study on the antibacterial activity of the products is still in progress.

CONCLUSIONS

Maleic acid units/oligomers have been grafted onto chitosan backbone by CAN initiation. The products were found to be soluble or swell

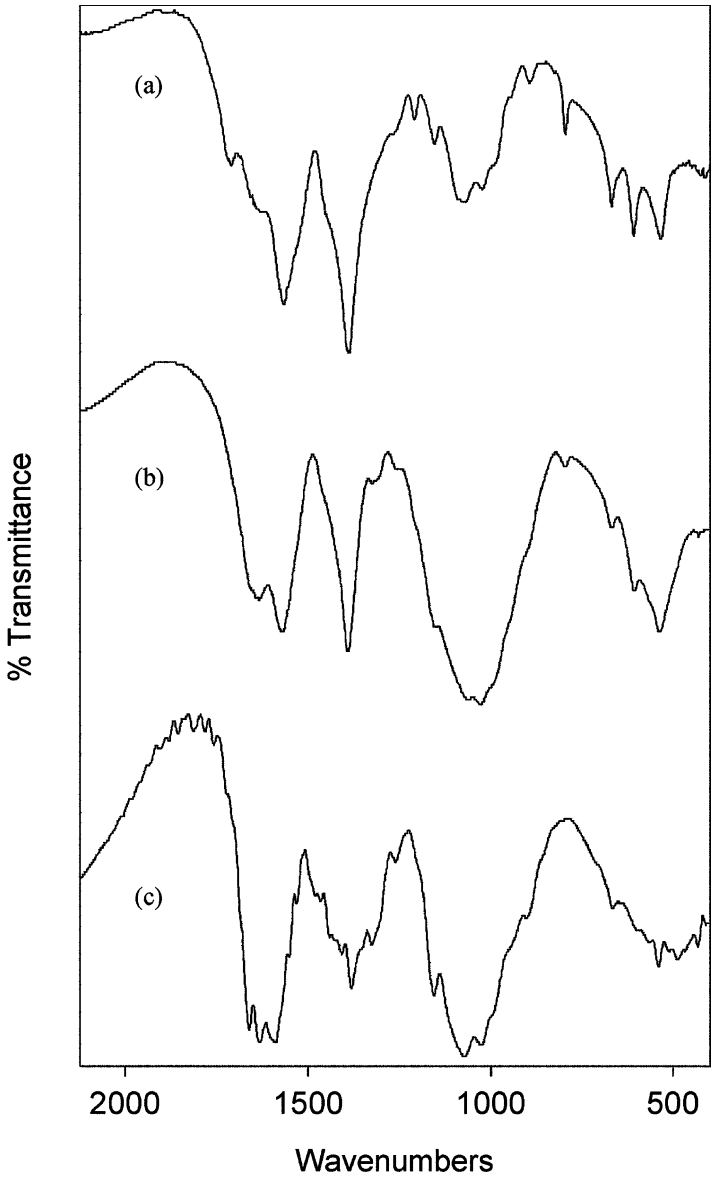

Figure 8. FTIR spectra of maleic acid grafted chitosan (sample 8); (a) swollen in pH 3 buffer and dried, (b) swollen in pH 7 buffer and dried, (c) swollen in pH 11 solution and dried.

in aqueous solution depending on the percentage of grafting. Both soluble and insoluble products are potentially useful for pharmaceutical applications.

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