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Graft Copolymerization of 2-Hydroxy Ethyl Methacrylate onto Chitosan with Cerium (IV) Ion. I. Synthesis and Characterization

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ABSTRACT

Graft copolymerization of 2-Hydroxy ethyl methacrylate (HEMA) on to chitosan was studied using cerium (IV) as the initiator. Optimization of the grafting was worked out by varying the reaction time and monomer concentration. Under controlled conditions, up to 685% grafting with a grafting yield of 92.4% was achieved. FTIR, thermal and XRD techniques were used to confirm the formation of the grafted copolymer. Grafting caused a marginal decrease in the mechanical strength in the dry conditions and a significant decrease under wet conditions for the resultant polymer. The products showed significantly improved swelling at pH 7.4 and pH 1.98 compared to the original chitosan. Grafted polymer showed enhanced Tg and decomposition temperature. The grafting also resulted in improved hydrophilicity as is evident from the contact angle studies of the films.

Key Words: Chitosan; Graft copolymer; HEMA; Hydrophilic polymer.

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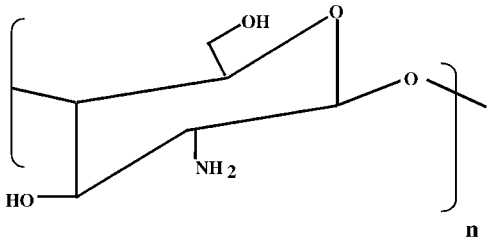
INTRODUCTION

Chitosan [β-(1–4)-2-amino-2-deoxy-D-glucose] is a unique basic polysaccharide and is generally represented as a homopolymer.



CHITOSAN

Most other commercial polysaccharides are either neutral or acidic. Chitosan occurs in nature in the cell walls of some fungi, insects, etc. It is prepared from chitin by N-deacetylation with 40–50% aqueous alkali.[1,2]

The deacetylation process is rarely complete and most commercial and laboratory products tend to be copolymers with N-acetyl glucosamine and N-glucosamine as repeat units. Chitosan is soluble only in aqueous acidic solutions such as acetic and hydrochloric acids via protonation of amine functions. The solubility depends upon the distribution of free amino groups and N-acetyl groups.[1]

Chitosan has been shown to be a reactive crystalline polysaccharide and is noted as a functional polymer with applications in various fields. It is non-toxic and biodegradable[3] which has increased its applicability in the pharmaceutical and biomedical fields.[4–6] This speciality polysaccharide, having specific properties such as biodegradability, biocompatibility, and bioactivity has invited a great deal of research interest. These properties make chitosan a suitable product for biomedical applications such as in wound dressings,[7] blood anticoagulant,[8] carrier for controlled release of drugs,[9] functional membranes, space filling implants,[10,11] etc. It seems to fulfill a number of demands in the highly technological world. Chemical modification may become a breakthrough to promote utilization of chitosan and among them, graft copolymerization is anticipated to be a promising approach providing a wide variety of molecular design.[12] The properties of chitosan can be properly tuned by the nature and concentration of the graft.

Reports on the graft copolymers of chitosan with vinyl monomers are very scarce.[13,14,21] Much of the work focuses on the physical blend of chitosan with other polymers due probably to the difficulty in effecting the grafting,. Thus, blends of chitosan with acrylic acid,[15] polyethylene glycol,[16] poly(vinylalcohol),[18,22] etc. have been reported. Such blends find speciality application in the biomedical field. Poly (HEMA) is a hydrophilic polymer, known to be biocompatible. It is of interest to synthesize a graft copolymer of chitosan with HEMA and evaluate its properties for assessing its suitability in biomedical applications. An earlier work[19] refers to the grafting of HEMA on chitosan by a γ-irradiation technique. This method could lead to uncontrolled grafting and possible crosslinking. The present paper concerns a free radical technique for synthesizing the graft copolymer.

Materials

Chitosan was received from India Sea Foods, Cochin. Ceric ammonium nitrate was received from E. Merck and was used as is. 2-Hydroxy ethyl methacrylate (HEMA, Merck) was purified by vacuum distillation.

Grafting

Chitosan was dissolved in 2% aqueous acetic acid by stirring for 48 h at room temperature to make a 2% solution. 100 ml of this solution was placed in a three-necked round bottomed flask, fitted with a condenser and stirrer. 0.1 M Ceric Ammonium Nitrate (CAN) in 10 ml of 1 N nitric acid was used as the initiator. The reaction was carried out at 70°C for 5 h under nitrogen atmosphere with constant stirring. Then, the product was precipitated by adding 30 ml of 10% sodium hydroxide with vigorous stirring. The precipitate was washed with distilled water several times and filtered. The homopolymer was removed from the grafted product by soxhlet extraction using methanol until a constant weight was obtained for the product and also until the methanol extract was devoid of free HEMA as confirmed by FTIR as follows. 1 g of the already extracted chitosan-HEMA graft copolymer was again extracted with 50 ml of methanol for 5 h. The filtrate was concentrated and spread on a KBr window to make a thin film. The methanol was evaporated with the help of an IR lamp. The FTIR spectrum of the thin film was recorded by running a background spectrum with empty KBr window. The FTIR spectrum should not contain any characteristic peak at or around 1720 cm^{1} representative of the —C═O-peak of the homopolymer poly (HEMA).

The experiments were repeated by regulating the concentration of the monomer and reaction time. The different compositions were coded as CH-0, CH-5, CH-7.5, CH-10, and CH-12.5. (see Table 1).

Percentage yield was calculated by the following equation:

$$\text{Percentage yield} = \frac{\text{Weight of the graft copolymer}}{\text{Weight of chitosan} + \text{Weight of HEMA}} \times 100$$

The percentage grafting was calculated using the equation:

$$\text{Percentage grafting} = \frac{\text{Weight of the graft copolymer} - \text{Weight of chitosan}}{\text{Weight of chitosan}} \times 100$$

The monomer conversion was calculated with the following equation.

$$\text{Percentage conversion} = \frac{\text{Weight of the graft copolymer} - \text{Weight of chitosan}}{\text{Weight of Monomer}} \times 100$$

Preparation of Films

The grafted product was dissolved in 2% aqueous acetic acid with continuous stirring for 2–3 days. The solution was then poured into disposable polystyrene molds and cured at

Table 1. Graft copolymerization of HEMA onto chitosan with cerium (IV).

No.	Wt. of chitosan (g)	Wt. of HEMA (g)	Amount of CAN (in10 ml, 1 $NHNO_3$)	Time (hr)	Wt. of the product (g)	Monomer conversion (%)	Monomer conversion rate (%/min)	Yield (%)	Grafting (%)	Polymer reference
1	2	10	0.1 M	3	4.90	29	0.16	40.7	144	CH-10
2	〃	〃	〃	4	6.50	45	0.19	54.4	226	〃
3	〃	〃	〃	5	9.70	77	0.26	81.0	385	〃
4	〃	〃	〃	7	9.60	76	0.18	80.0	380	〃
5	〃	5	〃	5	4.40	48	0.16	63.3	121	CH-5
6	〃	7.5	〃	5	6.50	60	0.20	68.7	225	CH-7.5
7	〃	12.5	〃	5	10.50	68	0.23	72.4	425	CH-12.5
8	〃	15	〃	5	15.70	91	0.30	92.4	685	CH-15
9	〃	25	〃	5	15.00	52	0.17	57.0	650	CH-25

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45–50°C for 48 h. The formed film was neutralized with 2% sodium hydroxide solution. It was then removed from the dish and extensively washed with distilled water to remove residual sodium hydroxide and stored in distilled water until use.

Infrared Spectral Analysis

FTIR spectra were studied using Nicolet Impact 410 FT-IR Spectrometer. The FTIR spectral analyses were utilized to prove grafting. For this purpose, the FTIR spectra of Chitosan, poly (2-Hydroxy ethyl methacrylate) and the grafted copolymers of chitosan and HEMA were taken in the range 600–4000 cm^{-1}.

Thermal Studies

Glass transition temperature (Tg) of grafted films was measured by differential scanning calorimetry (DSC 2920 Differential Scanning Calorimeter, TA Instruments Inc.) and thermal decomposition temperatures of the films were studied on a SDT 2960, (Simultaneous TGA-DTA, TA Instruments Inc.).

Mechanical Properties

Tensile properties of the grafted films were studied using Universal Testing Machine-Instron 1193. The films were conditioned in the testing atmosphere for 48 h. Rectangular strips of 10-mm width were cut and thickness measured using a micrometer. Full-scale load range of 1 KN was applied at a cross head speed of 10 mm/min. Stress at break, percentage elongation and the modulus were calculated. The computed values are the mean of 6 repeat measurements.

Swelling Properties

Films of dimension $10 \times 10\,mm^2$ size films of known weight were immersed in phosphate buffer of pH 7.4 and aqueous acetic acid of pH 1.98 for known intervals of time. The pieces were removed carefully, blotted between filter paper to remove excess fluid and weighed.

$$\text{Swelling Index} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Contact Angle Study

Octane in water contact angle of the chitosan and the grafted films were determined using a contact angle goniometer (GII, Kernco Instruments Co. Inc.) to study

the hydrophilicity of the films. The films were incubated by putting in doubly distilled water for 3 days after cleaning thoroughly with distilled water. The films were then placed on glass slides and fastened on both ends using Teflon tapes. The slide was then immersed in a Perspex tank containing doubly distilled water. The octane droplets were introduced on the surface of the films in water using a micro syringe having a bent needle. The octane/water contact angles were then measured.

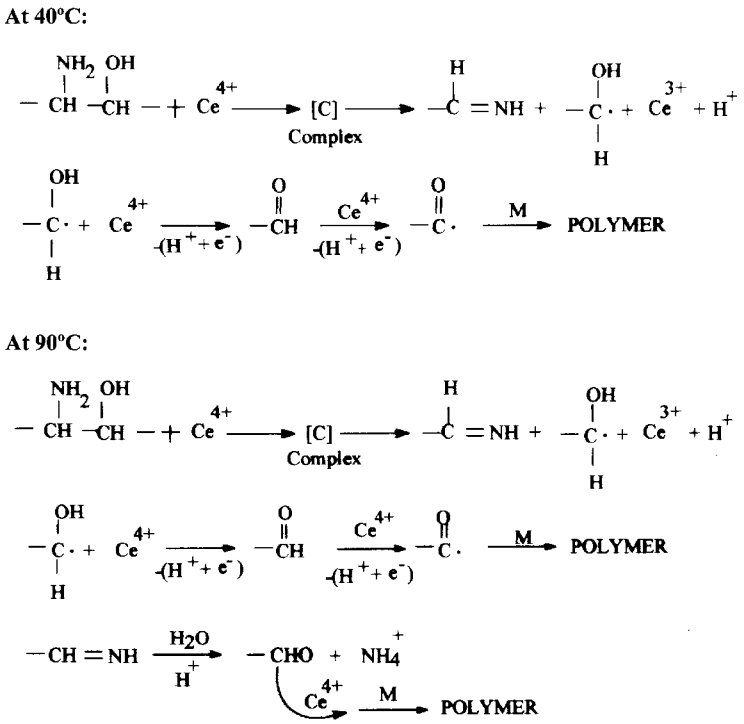
X-Ray Diffraction Patterns

X-ray diffraction patterns of the chitosan and the grafted copolymers in the powder form were performed by a wide angle X-ray scattering using Siemens D5005 X-Ray Diffractometer.

RESULTS AND DISCUSSION

HEMA was grafted onto Chitosan via the Ce (IV) ion method. Gurruchaga et al. used a similar method to prepare HEMA-grafted starch.[17] Blair et al. have reported a method for the homogeneous grafting of chitosan and chitin with vinyl monomers such as methylacrylate, vinylacetate etc using AIBN as the initiator.[18] Reports on grafting HEMA onto chitosan are rare. Singh and Ray grafted HEMA onto chitosan using ^{60}Co gamma radiation and studied the blood compatibility of the products.[19] In this work, we used ceric ammonium nitrate in 1 N nitric acid as the initiator. Wei et al. proposed the following mechanism of initiation of graft copolymerization of vinyl monomers onto chitosan.[12] The chitosan forms an efficient redox initiation system with Ce ion, and a chelate complex is formed when Ce (IV) ion reacts with the adjacent hydroxyl-amino structure. At 40°C, the amino and hydroxyl groups are oxidized to —CH═NH and aldehyde groups, respectively. At higher temperature (~90°C) the —CH═NH group is also hydrolyzed to form aldehyde group that forms the reaction complex to initiate polymerization (Sch. 1).

In all experiments, 2 g chitosan dissolved in 2% aqueous acetic acid and 0.1 M ceric ammonium nitrate in 10 ml of 1 N HNO_3 were added. The reaction time was varied. Maximum yield was obtained at 5 h with 15 g of HEMA (92.4%). The extent of grafting was calculated after the removal of the homopolymer by soxhlet extraction with methanol. The complete removal of the homopolymer was confirmed by FTIR analysis of the extract as explained in the Experimental part. Enhancing the weight of HEMA up to 15 g led to a enhanced yield (Table 1). But when the weight of HEMA was increased further, say to 25 g, the % yield decreased to 57%. Grafting increased with reaction time. The yield % did not increase significantly beyond 5 h of reaction under the conditions employed here. The conversion was in the range of 48 to 91% for the different compositions. But the % graft did not increase on enhancing the monomer weight further up to 25 g. This is due to the fact that when the weight of HEMA in the medium becomes very high the solution becomes highly viscous and the active sites of chitosan may not be readily available for the grafting reaction to take place. The homopolymerization process exceeds the grafting reaction, thereby decreasing the graft %.



Scheme 1. Mechanism of cerium ion mediated grafting of HEMA at two temperatures.

The graft polymers were obtained as off-white powdery materials. The different compositions were coded as CH-5, CH-7.5, CH-10, and CH-12.5, respectively. A plot of apparent graft ratio as a function of the relative concentration of HEMA in the medium is shown in Fig. 1.

Infrared Spectral Analysis

The FTIR study was conducted to investigate the graft copolymerization between chitosan and HEMA. The grafting of HEMA onto chitosan was ascertained by the presence of the characteristic absorption peak of the carbonyl group at 1711 cm^{-1}. Typical spectra are given in Fig. 2. Amide I and Amide II bands of pure chitosan are located at 1643 cm^{-1} and 1552 cm^{-1}, respectively. The carbonyl band of the poly (HEMA) appeared at 1717 cm^{-1} On the other hand, the carbonyl absorption band of the HEMA–chitosan graft copolymer shifted to 1711 cm^{-1}. Ahn et al. noted a similar shifting of the carbonyl peak to lower frequency for poly acrylic acid–chitosan complex.[15] This is ascribed to the hydrogen bonding of the carbonyl with the amino and hydroxyl groups of chitosan.

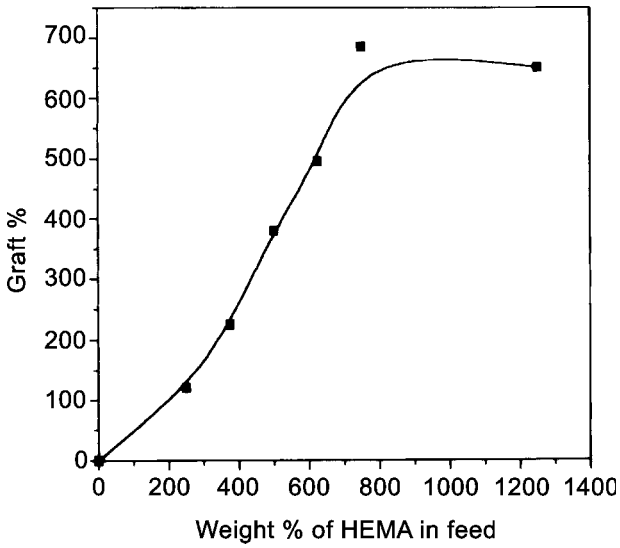


Figure 1. Dependency of grafting efficiency on monomer concentration in the feed.

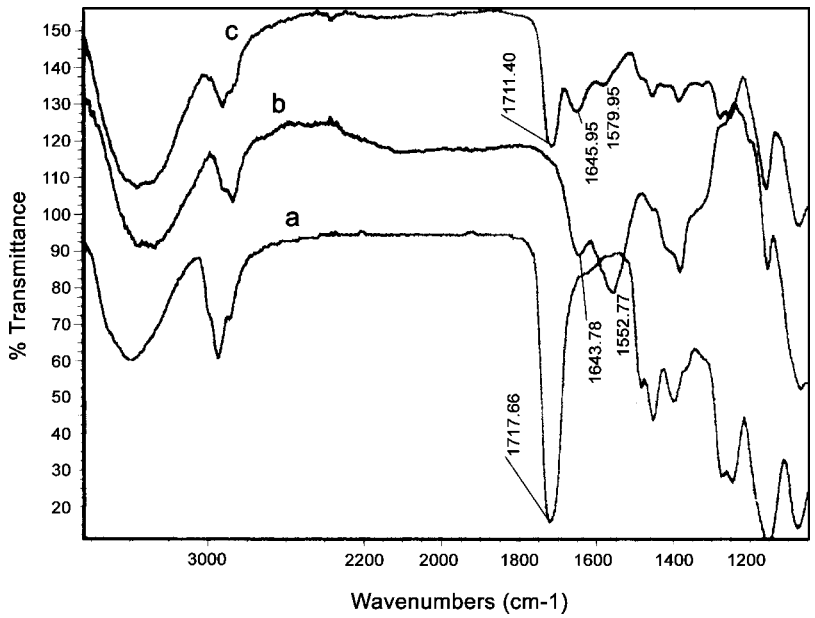


Figure 2. FTIR Spectra of a) poly (HEMA); b) chitosan; c) chitosan–HEMA copolymer.

Thermal Analysis

The glass transition temperature (Tg) of the graft copolymer was measured by differential scanning calorimetry. The values were taken from the second run after heating and cooling. The copolymer Tg is invariant with the extent of grafting beyond a grafting of 225%. This is shown in Fig. 3. Grafting led to enhanced Tg. This implies a stronger intermolecular interaction in the copolymer between the graft and the core, possibly by way of H-bonding. Zhang et al. studied the variation of glass transition temperature of chitosan blended with PEG[20] and observed little difference between chitosan and its blend. Don et al. could not observe any glass transition in the DSC thermograms of chitosan because of its highly rigid chains.[21] In the present study, we got a very distinct Tg for chitosan in the second heat cycle at 82.5°C and this value increased with the extent of grafting (Fig. 3). The graft copolymers showed a single Tg indicating the formation of a homogeneous matrix containing the chitosan core and poly (HEMA) grafts. The Tg values in between those of chitosan and poly (HEMA) confirmed the miscibility of the segments.

The grafting with HEMA enhanced the thermal stability of chitosan as seen from the TGA of the polymers. In pure chitosan, the first decomposition temperature was observed at 248°C with 83% weight remaining at this stage. At 582°C, 36% of the chitosan residue remained. The TGA of chitosan, poly (HEMA) and the copolymers of varying compositions are shown in Fig. 4. It is seen that the copolymer undergoes a two stage decomposition corresponding to the core and the graft. However, the overall decomposition temperature of the graft copolymers are elevated vis-à-vis pure chitosan. This is attributed to the thermal

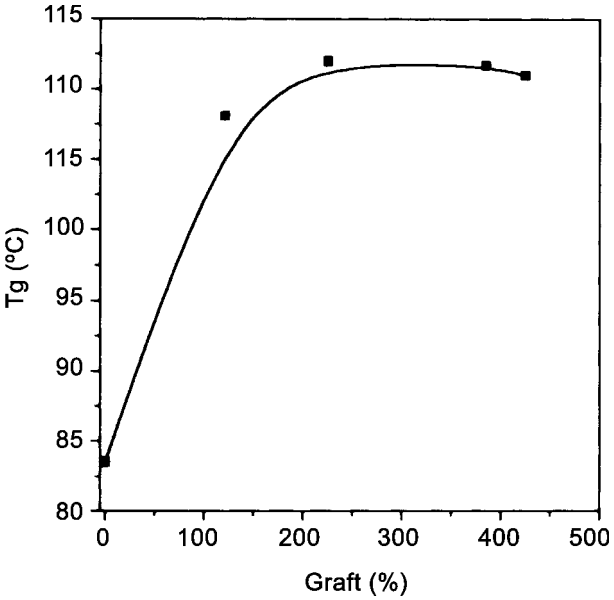


Figure 3. Effect of grafting on glass transition temperature.

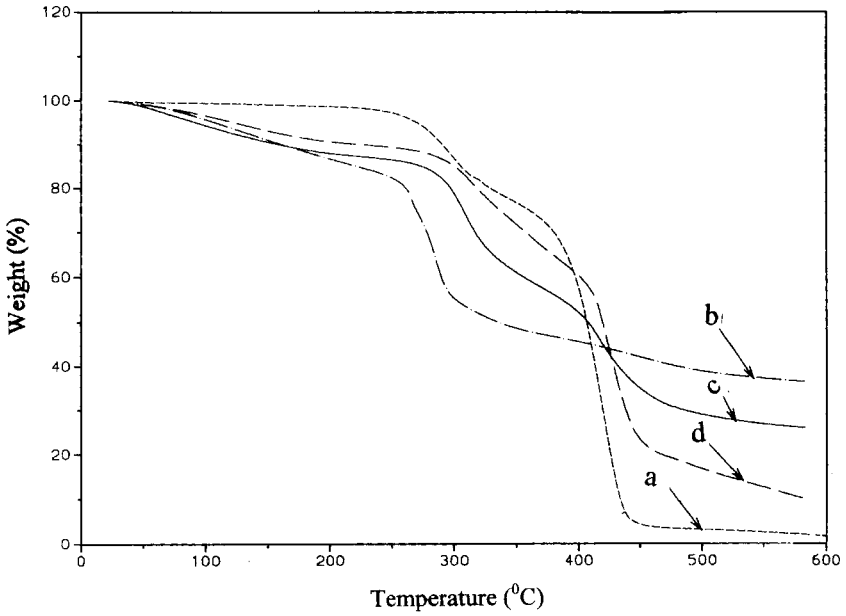


Figure 4. TGA thermograms of a) poly (HEMA); b) chitosan; c) CH-7.5; and d) CH-12.5. (Under nitrogen atmosphere at a heating rate of 20°C/min).

stabilization of the core-ring by way of H-bonding with the pendant grafts. The second stage decomposition occurring at ~420°C is attributed to the HEMA segments as this is comparable to the thermogram of pure poly (HEMA). The thermal decomposition data are compiled in Table 2. Grafting resulted in an increase in the initial decomposition temperature (T_i) and the temperature corresponding to 50% decomposition(T_{50}). As the HEMA content increases, the char residue of the graft copolymer decreases at the maximum temperature T_{max}. This implied a decreased thermal stability of the graft copolymer at elevated temperatures. The corresponding DTG of all the grafted films confirmed the two stage decompositions. The peak around 415°C is due to the decomposition of poly (HEMA) segment which is proved from the DTG of pure poly (HEMA) Fig. 5.

Mechanical Properties

It is seen from Table 3 that the grafting results in a decrease in the tensile strength of chitosan, particularly beyond about 425% grafting. The elongation also decreases. HEMA leads to rigidification of the graft copolymer by way of strong intermolecular H-bonding. Under wet conditions, grafted polymers showed a further decrease in property. Hydrogen bonding with water breaks down intermolecular interaction through hydrogen bonding among the polymer molecules. This plasticization effect is reflected in a higher elongation in the wet condition for the graft copolymer. The modulus values of the films also show

Table 2. The effect of grafting on the decomposition temperature of chitosan.

Films	T_i	Residue (%)	T_{50}	T_{max}	Residue (%)
CH-0	248	83	335	582	36
CH-5	261	87	413	584	25
CH-7.5	268	85	408	583	26
CH-10	292	85	422	583	15
CH-12.5	268	89	420	582	9.8

a significant decrease under wet conditions which again confirms the diffusion of water molecules through the copolymer matrix to decrease the strong intermolecular forces between the core and the graft. In the case of virgin chitosan, the plasticization effect of water enhances its strength and elongation. The elastomeric nature of wet chitosan film is reflected from its decreased modulus values in the wet state in comparison to that of the dry state.

Some studies report that blending or grafting of chitosan with hydrophilic monomer decrease the mechanical property of the chitosan film. Zhang et al. found that when the PEG concentration is increased, the mechanical properties of the chitosan-PEG blends deteriorated.[20] On the other hand, Chandy et al. reported improved

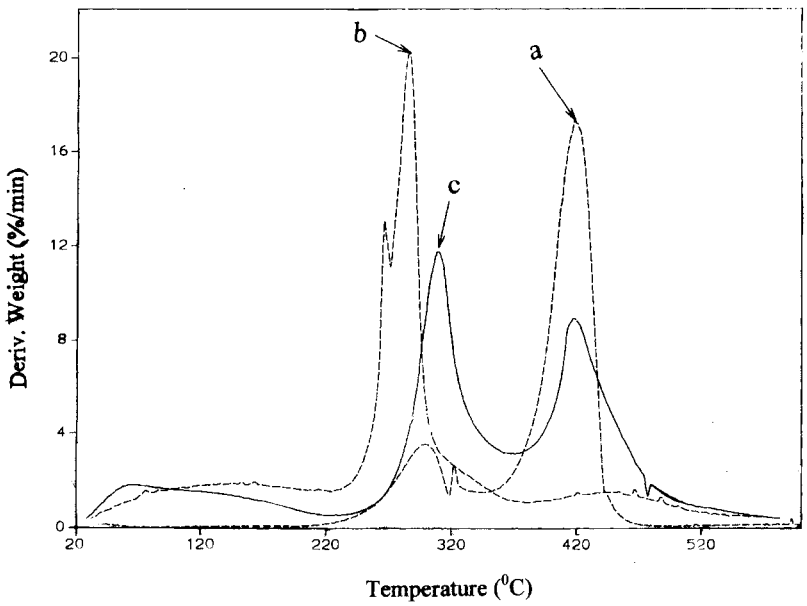


Figure 5. DTG thermograms of a) poly(HEMA), b) chitosan, c) chitosan–HEMA copolymer.

Table 3. Mechanical properties of the grafted films.

	Dry			Wet		
Sample code	Tensile strength (MPa)	Elongation (%)	Modulus (MPa)	Tensile strength (MPa)	Elongation (%)	Modulus (MPa)
CH-0	34.5 ± 11.2	14.9 ± 7	1477 ± 1050	51.1 ± 6.8	92.3 ± 6.8	71 ± 14
CH-5	33.1 ± 2.7	2.9 ± 0.4	1529 ± 560	7.9 ± 3.3	89.7 ± 25.6	141 ± 93
CH-7.5	29.0 ± 6.9	6.4 ± 3.4	1398 ± 604	12.3 ± 1.9	55.1 ± 4.5	30 ± 3.0
CH-10	33.6 ± 8.3	2.0 ± 0.2	2346 ± 146	17.6 ± 2.8	89.9 ± 12.6	88 ± 10
CH-12.5	22.1 ± 0.2	3.2 ± 1	1020 ± 322	12.8 ± 0.6	65.0 ± 2.7	84 ± 13

mechanical properties for Chitosan–PVA blends under wet conditions[22] Blair et al. reported that when chitosan is blended with PVA, none of the blend membranes was as strong as the chitosan or the poly(vinyl alcohol) membranes.[18] A small amount of PVA in the blend produced a large reduction in strength and elasticity of chitosan. Our observation is similar to that of Blair who also used a very hydrophilic monomer (Vinyl alcohol) like HEMA in the present study. It may be concluded that grafts of hydrophilic polymers lead to deterioration of mechanical strength particularly in wet conditions.

Hydrophilicity of Graft Copolymers

The swelling characteristics of the grafted polymers were studied at pH 7.4 and at pH 1.98. At pH 7.4, the swelling increased to a maximum of 93% (with respect to chitosan) during 1 h in the case of CH-12.5. At pH 1.98, it increased considerably with respect to chitosan for the same composition when studied for 10 min. The swelling response of chitosan and the grafted films at pH 7.4 and 1.98 are shown in Figs. 6a and 6b, respectively. The swelling reaches a stable equilibrium much more rapidly at pH 7.4 than at pH 1.98. The swelling % of the films at pH 1.98 could not be determined accurately beyond 10 min, as the films started degrading at this stage. This indicated that the dissolution tendency of the films exceeded the swelling. A similar behavior was observed by Gupta et al. while studying the pH dependency of the hydrolysis of the chitosan/PEG polymer network microspheres.[16] The observed swelling of the grafted films increases with the extent of grafting. The increase in swelling could be due to the high diffusivity of water into poly (HEMA) matrix. The % swelling of the pure chitosan film is significantly less at pH 7.4, which shows its inherent hydrophobicity at high pH value. Swelling studies imply that the hydrophilicity is much improved by graft co-polymerization of HEMA onto chitosan. This is further confirmed from the octane contact angle studies.[23] As the percentage grafting increases, the contact angle increases implying a proportionally improved hydrophilicity Fig. 7.

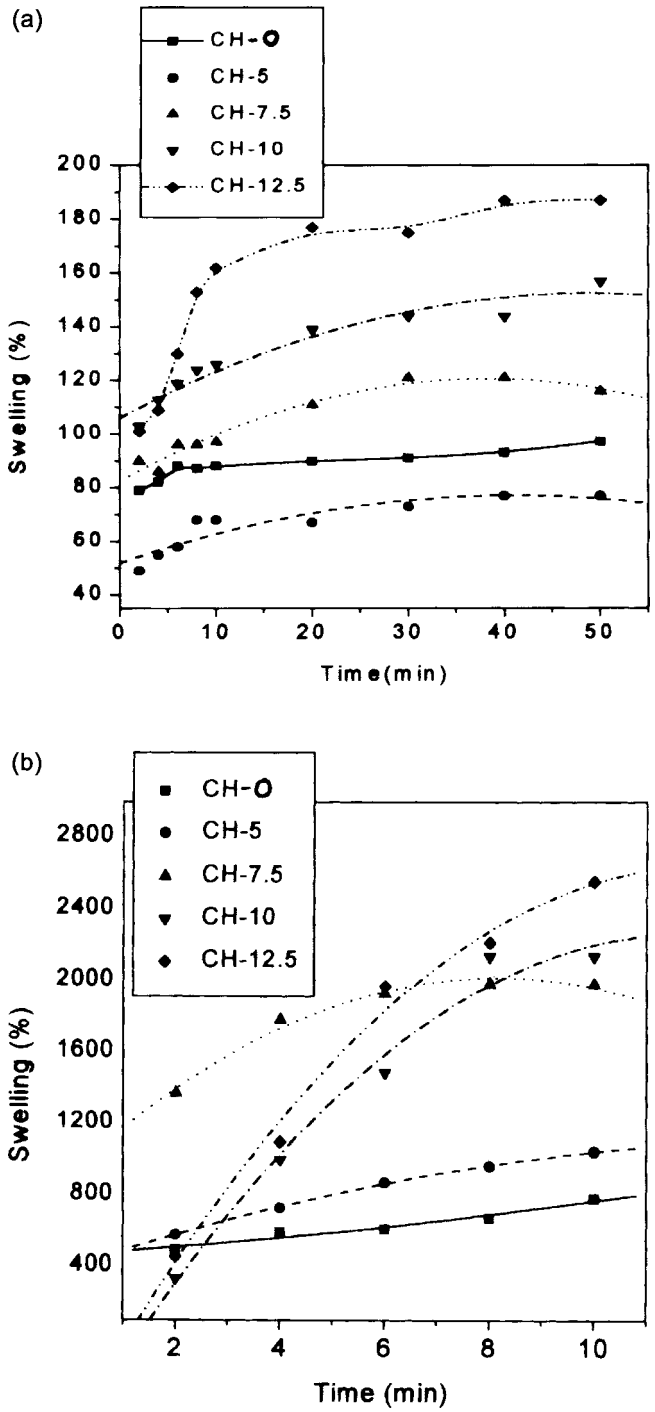


Figure 6. a) Swelling properties of the grafted films at pH 7.4. b) Swelling properties of the grafted films at pH 1.98.

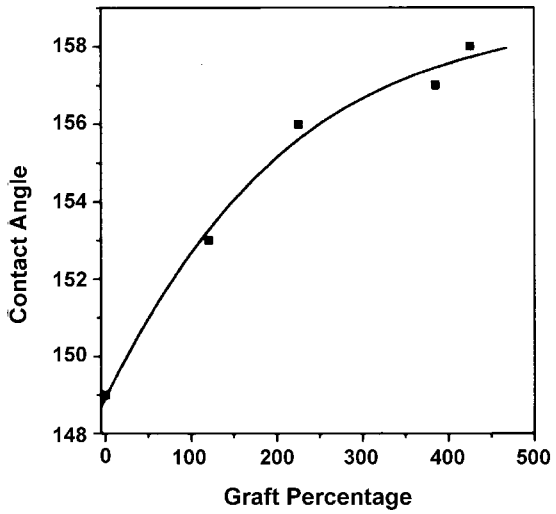


Figure 7. Octane contact angle variation of the films with % grafting.

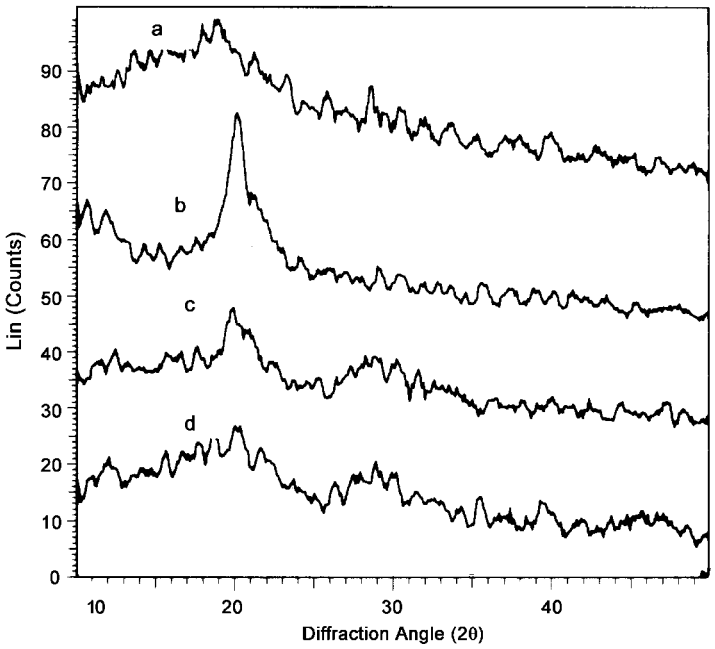


Figure 8. XRD Pattern of a) poly (HEMA); b) chitosan; c) CH-7.5; and d) CH-12.5. (X-ray powder diffraction method).

X-Ray Diffraction Patterns

Wide angle X-ray diffraction patterns of powdered poly-HEMA, chitosan and the graft co-polymers are shown in Fig. 8. Chitosan exhibited a typical peak that appeared at $2\theta \sim 20$. These peaks were assigned to a mixture of the planes (001) and (100) of (101) and (002), respectively.[24] The intensity of this peak decreases with the increase in concentration of HEMA in the medium. When chitosan-HEMA graft copolymers are formed in the presence of Ce (IV), the amino and hydroxyl groups form a complex to initiate polymerization. This may break the hydrogen bonding between amino and hydroxyl groups in chitosan and this results in the amorphous structure of the graft copolymer. The decrease in the intensity of the peaks indirectly implied grafting of HEMA on chitosan.

CONCLUSION

HEMA was successfully grafted onto chitosan with ceric ammonium nitrate as initiator under controlled conditions. Grafting was enhanced by HEMA concentration in the medium. The graft copolymerization was confirmed by FTIR, thermal and XRD studies. The Tg of the grafted copolymer was found to be in between the Tg of chitosan and poly-(HEMA). The grafted products showed good film forming properties. Grafting resulted in rigid polymer whose mechanical properties in wet conditions were significantly impaired due to the plasticizing effect of water. The grafted films showed enhanced hydrophilicity and overall thermal stability compared to chitosan. The copolymers showed very high swelling in acid pH and limited swelling in physiological pH which are essential requirements for developing gastrointestinal drug delivery systems using these polymers. The properties such as biocompatibility, biodegradability and diffusivity of the films remain to be studied for confirming their clinical applicability.

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