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Carbohydrate Polymers 54 (2003) 343–351
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Studies on graft copolymerization of chitosan with synthetic monomers

K.V. Harish Prashanth, R.N. Tharanathan*

Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570 013, India

Received 5 February 2003; revised 27 June 2003; accepted 30 June 2003

Abstract

Graft copolymerization of acrylonitrile and methylmethacrylate onto chitosan using potassium persulfate as an initiator was studied. Evidence for graft-copolymerization was obtained by infrared spectroscopy and CP-MAS ^{13}C-NMR data. The appearance of nitrile (–C≡N) at 2244 cm^{-1} for chitosan-graft-polyacrylonitrile (C-*g*-PAN) and carbonyl (–C=O) at 1730 cm^{-1} for chitosan-graft-polymethylmethacrylate (C-*g*-PMMA) confirmed graft-copolymerization. CP-MAS ^{13}C-NMR showed the appearance of a signal at 33 ppm assigned to the N–CH group. With varying monomer concentration (40–180 mM), the percentage degree of substitution varied from 2 to 50. Maximum grafting efficiency was obtained with 120 mM acrylonitrile and 0.74 mM potassium persulfate at 65 °C for 2 h under nitrogen atmosphere for 1% chitosan solution and for C-*g*-PMMA 140 mM methylmethacrylate at 75 °C gave maximum substitution. X-ray diffraction showed changes in crystallinity pattern. Slightly different mechanisms in side chain substitution for these two copolymers were envisaged. DSC thermogram showed a decomposition peak for C-*g*-PAN at around 255 °C and a melting peak for C-*g*-PMMA at around 400 °C. C-*g*-PMMA could be thermopressed into films. Residual monomers were not found by HPLC in graft copolymers stored even for longer periods.

Keywords: Chitosan; Polyacrylonitrile; Polymethymethacrylate; Copolymer; Grafting

1. Introduction

Chitin is one of the most abundant biopolymers found in the shells of crustacea, e.g. crab and shrimp, and cuticles of insects and also in the cell walls of some fungi and microorganisms. Chitin consists of *N*-acetyl-D-glucosamine repeating units, linked by β-(1 → 4) bonds. Due to its inherent intractability, it is often converted to chitosan, 2-amino-2-deoxy-(1 → 4)-β-D-glucan by hot alkali treatment. Chemical modification of chitosan is an important topic for the production of bifunctional materials. Work on graft copolymer synthesis based on chitin and chitosan and their application has been reported. Chitosan-*g*-poly(glycidyl-methacrylate) copolymer was used for immobilization of urease (Chellapandian & Krishanan, 1998) and other modifications such as the grafting of methylmethacrylate onto chitin initiated by tributylborane (Kojima, Yoshikuni, & Suzuki, 1979), poly(3-hydroxyalkonate)-chitosan conjugates (Yalpani, Marchessault, Morin, & Monosteries, 1991), chitosan modified poly(glycidylmethacrylate–butylacrylate) and chitosan grafted bovine pericardial tissue-anticalcification properties have been studied (Shanthi & Panduranga Rao, 2001). However, modification of chitosan via grafting of vinyl monomers is one of the most effective methods to incorporate desirable properties into chitosan without sacrificing its biodegradable nature.

In the present work, the results of a study on grafting of acrylonitrile (AN) and methylmethacrylate (MMA) onto chitosan in terms of different monomer concentrations and using potassium persulfate as a free radical initiator, under innert conditions are described. Further, the effect of grafting on the thermal behaviour and structural aspects of chitosan have been examined. Attempts were made to prepare chitosan membranes out of graft copolymers and also check for any residual monomers using HPLC.

2. Experimental

2.1. Preparation of graft copolymers of chitosan

Chitosan, as a solution in dilute acetic acid was graft copolymerized under homogeneous conditions by the method of Chellapandian and Krishanan (1998) with the following modifications.

* Corresponding author. Tel.: +91-821-514876; fax: +91-821-517233.
E-mail address: tharanathan@yahoo.co.uk (R.N. Tharanathan).

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doi:10.1016/S0144-8617(03)00191-7

2.1.1. Chitosan graft-polyacrylonitrile

Chitosan solution (1.0%) was placed in a flat bottomed three necked flask. Throughout the reaction time, nitrogen was purged at a constant temperature (60 °C) through the stirred solution. Freshly prepared potassium persulfate solution (0.74 mM) was added followed by dropwise addition (40–140 mM) of acrylonitrile. The reaction was conducted for 2 h with stirring continued for another 15 min at room temperature. Then the reaction product was precipitated out with 2 vol of isopropanol, filtered, washed with distilled water, to remove the unmodified soluble low molecular weight chitosan (Harish Prashanth et al.—unpublished data) and dried. It was again subjected to Soxhlet refluxing for 8–12 h using *N*,*N*-dimethylformamide (DMF) to solubilize and remove the homopolymer, if any and finally lyophilized.

2.1.2. Chitosan graft-polymethylmethacrylate

To chitosan solution (1.0%) were added 0.74 mM potassium persulfate and varying concentration of methylmethacrylate (40–160 mM) and stirred continuously at 75 °C. The reaction was continued as previously described for C-*g*-PAN. The copolymer was extracted with acetone and thoroughly washed with distilled water and lyophilized. The gain in weight of the copolymer is measured as percent grafting $= W_g - W_0 \times 100/W_0$, where W_g is the weight in grams of the grafted chitosan and W_0 is the weight of native chitosan (Zhang & Chen, 2001).

2.2. Infrared spectroscopy

IR spectra were recorded in KBr discs on a Perkin Elmer Spectrum 2000 FTIR spectrometer under dry air at room temperature. Approximately 6 mg of dried sample was blended with 200 mg of potassium bromide (IR grade) and about 40 mg of the mixture was used to prepare a pellet.

2.3. Solid state CP-MAS ^{13}C NMR

Approximately 300 mg of freeze dried sample were inserted into a 7 mm ceramic rotor on a Bruker DSX 300 spectrometer. The spectra were recorded at 75.5 MHz. The crosspolarization pulse sequence was utilized for all samples, which were spun at the magic angle at 4 kHz. A contact time of 1 ms and a pulse repetition time of 5 s were used and more than 2500 scans were accumulated for each sample.

2.4. Scanning electron microscopy

The dry sample, spread on a double sided conducting adhesive tape, pasted on a metallic stub, was coated (100 μ) with gold in a sputter coating unit for 2 min and observed in a LEO-435-VP (LEO Electron Microscopy Ltd, Cambridge, UK) electron microscope at 20 kV.

2.5. High performance liquid chromatography

Separation of monomers was performed on a reverse phase Shim-Pack C18 (ODS) column (Shimadzu, Japan, 15 cm × 4.6 mm i.d.) in a Shimadzu HPLC system consisting of an LC 6A pump equipped with UV-Vis spectrophotometric detector. Residual monomers of graft copolymers were analyzed by HPLC according to the procedure of Saroja, Gowda and Tharanathan (2000), which involves separation using 0.05 M KH_2PO_4, pH 5.5 at 1 ml min^{-1} at 30 °C. A 10 μl volume of standards AN (acrylonitrile), AM (acrylamide), AC (acrylic acid) and MMA (methylmethacrylate, diluted in glass distilled water) were injected onto the column and detected at 220 nm. Their minimum detection limit varied from 1 to 10 ng.

2.6. Differential scanning calorimetry

The samples were analyzed using a Rheometric Scientific (UK) equipment supported by thermal software S42 on a Compaq computer, which is precalibrated. The equipment was provided with an autocool system. Accurately weighed (5 mg) material was placed in an aluminium cup and hermetically sealed. Empty sealed cup was used as reference and runs were performed in duplicates. Analyses were done under continuous flow of dry nitrogen gas (10 ml min^{-1}) at a heating rate of 20 °C min^{-1} from 5 to 500 °C.

2.7. X-ray diffractometry

Powder X-ray diffraction patterns of chitosan and copolymers were obtained by using a EG-7G solid state germanium liquid nitrogen cooled detector Sintag XDS-2000 instrument equipped with a $\theta-\theta$ goniometer, under the following operating conditions; 30 kV and 25 mA with Cu $K\alpha_1$-radiation at λ 1.54184 Å. The relative intensity was recorded in the scattering range (2θ) of 4–60°.

2.8. Membrane preparation

The purified chitosan graft copolymers (1 g) were heat pressed in between Teflon sheets at 115 ± 2 °C at a pressure of 150 kg cm^{-2} for 20 min using polymer film making machine (Techno Search (Pvt.) Ltd, India), which has both temperature/pressure control and autocooling systems.

3. Results and discussion

Polymer grafting reactions provide the potential for significantly altering the physical and mechanical properties of the starting materials. Some polymer grafts have tendency to form thin membranous films, which may be

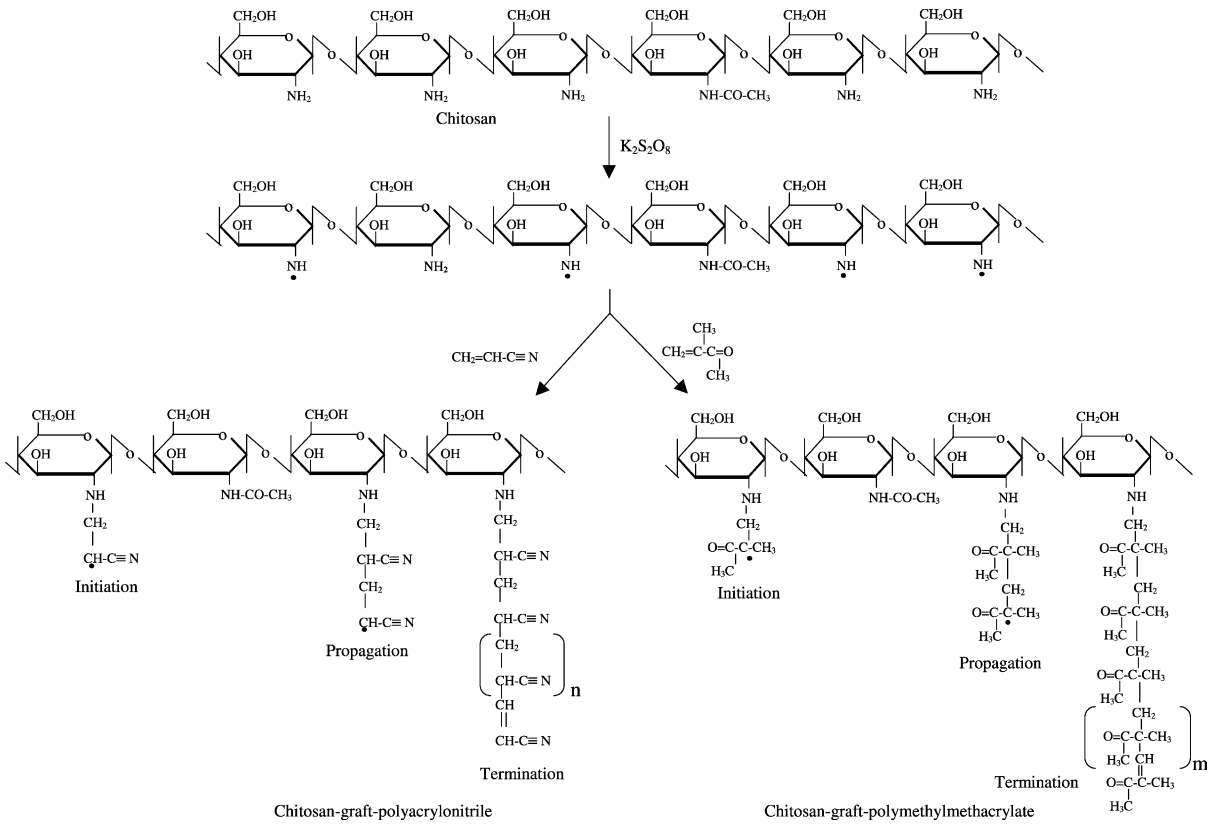

Fig. 1. Schematic representation of graft-copolymerization of chitosan.

useful for packaging applications. Polymer grafting is usually carried out by radical initiation, which is done either by photo induced free radical or by using free radical initiators. The main disadvantage in the former is the formation of homopolymers, because the UV light generates directly free radicals on vinyl monomers and thereby increasing homopolymerization, whereas with radical initiators the free radicals are produced on specific sites, which also increases the grafting efficiency. The reactive C-2 amino group in chitosan is important in several of the structural modifications targeted because the deprotonated amino group acts as a powerful nucleophile (p*K*a ≅ 6.3) readily reacting with electrophilic reagents (Chen, Kumar, Harris, Smith, & Payne, 2000). Even in free radical initiated copolymerization, NH_2 groups of chitosan involve in macroradical formation (Fig. 1). In the present investigation, potassium persulfate was used as a free radical initiator to induce grafting.

3.1. Characterization of the graft copolymers

From Table 1, it is obvious that the addition of side chains of PAN and PMMA to chitosan main chain (as shown in Fig. 1) leads to increase in weight due to copolymerization. The percent grafting was considerably more with C-*g*-PMMA than with C-*g*-PAN because of bulky methyl groups of PMMA in the former.

3.1.1. Solubility

The solubility characteristic of the material is another criterion for modification, since chitosan is soluble in water in the presence of H^+ ions (acetic acid, lactic acid or HCl),

Table 1
Different monomer concentrations, their percent grafting and decomposition/melting temperatures

Acrylonitrile concentration (mM)	Decomposition peak temperature (°C)[a]	Percent grafting (%)	Methylmethacrylate concentration (mM)	Melting peak Temperature (°C)[a]	Percent grafting (%)
40	255.02 ± 0.1	130	40	281.07 ± 0.2	161
60	259.65 ± 0.3	145	100	281.89 ± 0.1	165
100	ND	142	120	391.49 ± 0.3	273
120	302.10 ± 0.2	249	140	393.81 ± 0.5	276
140	255.00 ± 0.3	170	180	390.01 ± 0.1	271

ND, not determined.
[a] The values presented are average of two separate runs with standard deviation.

whereas the grafted chitosans were insoluble even in solvents like DMF and acetone, which are known to solublize PAN and PMMA homopolymers, respectively. In addition to the formation of graft copolymers, cross-linking between the chains of chitosan may also take place. This was evident by the reaction between chitosan and the initiator, in the absence of monomers, giving a product with reduced solubility (unpublished data). Variations in initiator concentration, reaction temperature and time did not show much effect on the yield and grafting efficiency, as compared to that observed in changing monomer concentration (Table 1). Maximum grafting efficiency was obtained for C-*g*-PAN with 120 mM acrylonitrile at 65 °C, and for C-*g*-PMMA with 140 mM methymethacrylate at 75 °C.

3.1.2. Infrared spectroscopy

Infrared spectroscopy is the best tool to confirm the grafting reaction. The appearance in C-*g*-PAN of-C≡N (nitrile) absorption at around 2244 cm^{-1} and the CH_2 deformation vibration at around 1453 cm^{-1} (Fig. 2a) and in C-*g*-PMMA of 1731 cm^{-1} due to –C=O (carbonyl) absorption and methyl and methylene asymetric stretching vibrations at 2995 and 2951 cm^{-1}, respectively, (Fig. 2b) confirmed grafting. With increase in percent grafting, the intensity of these absorption also increased (Fig. 3a and b).

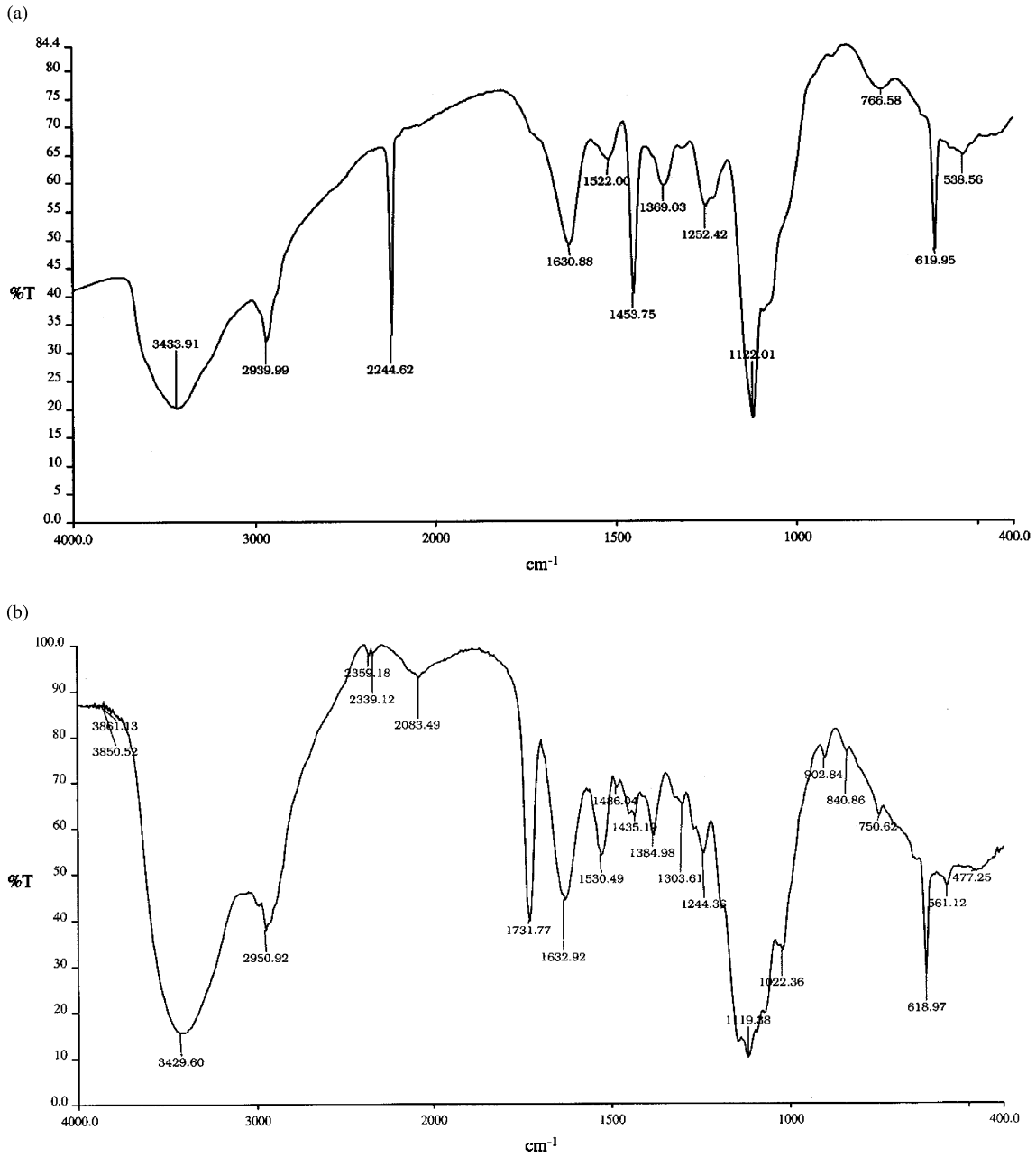

Fig. 2. IR spectra of (a) C-*g*-PAN, (b) C-*g*-PMMA.

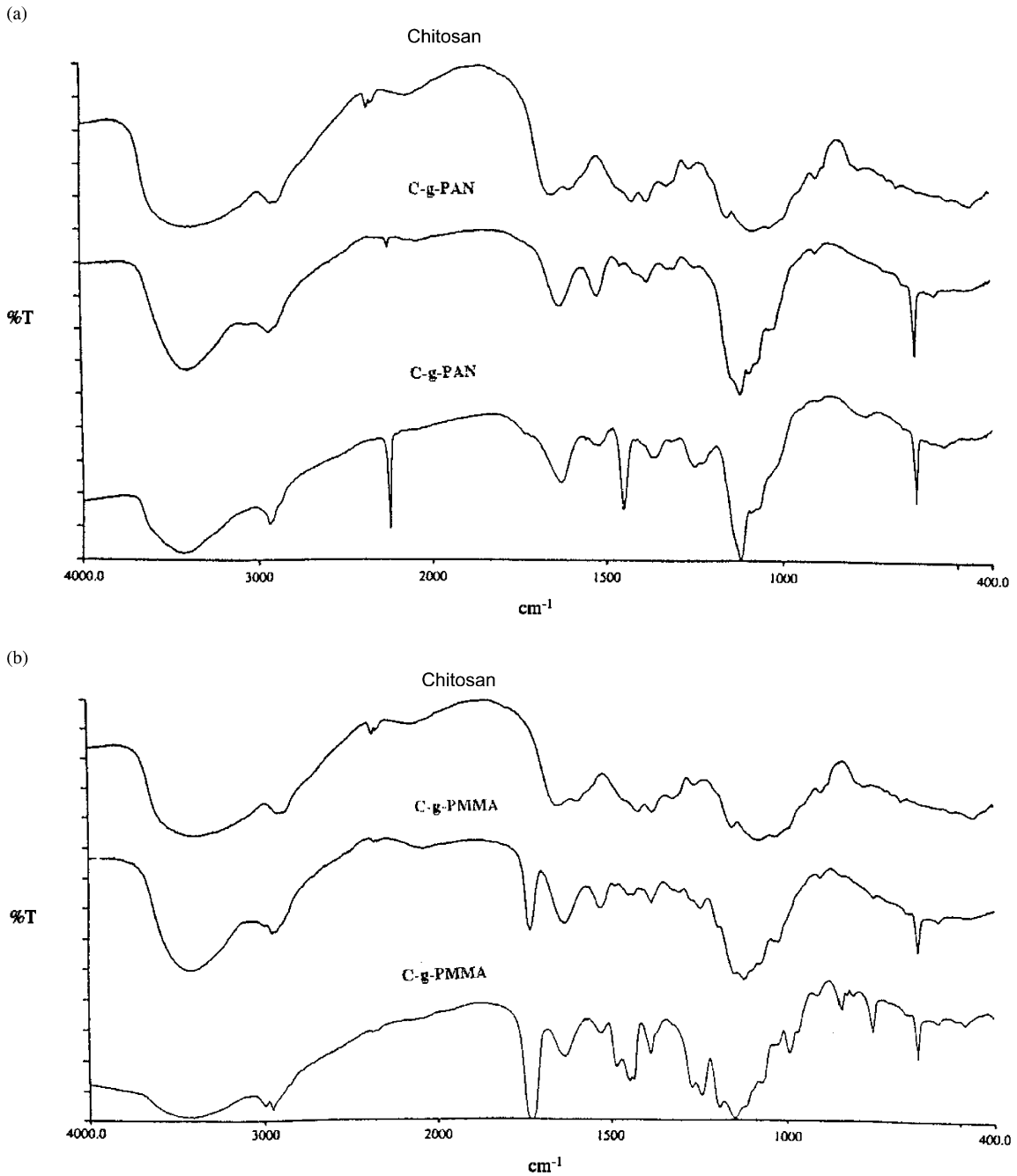

Fig. 3. IR spectra of graft-copolymers with increase in percent grafting (a) C-*g*-PAN, (b) C-*g*-PMMA.

The absence of a strong (sharp) absorption around 3500 cm^{-1} indicated the absence of free –OH groups of chitosan, which probably are involved in some hydrogen bonding. The region above 3000 cm^{-1} was centered at 3396 cm^{-1} for chitosan, which was shifted to a higher frequency for graft copolymers, indicating an increase in the ordered structure. The amide I and amide II absorptions were seen around 1630 and 1530 cm^{-1}, and with increase in percent grafting a progresssive weakening of amide II band was observed. The –C–O–C– stretching absorption for chitosan was seen at 1080 cm^{-1}, which was shifted to a slightly higher frequency with a sharp band (1120 cm^{-1}), probably due to change in crystallinity.

3.1.3. CP-MAS ^{13}C-NMR

Solid state NMR data indicated changes in the local order structure (Fig. 4). The line widths of chitosan in graft copolymer were relatively broadened because of the introduction of side chains. The magnitude of –N–CH signal (Capitani, Angelis, Crescenzi, Masci, & Segre, 2001; Rinaudo, Desbrieres, Le-Dung, Binh, & Dong,

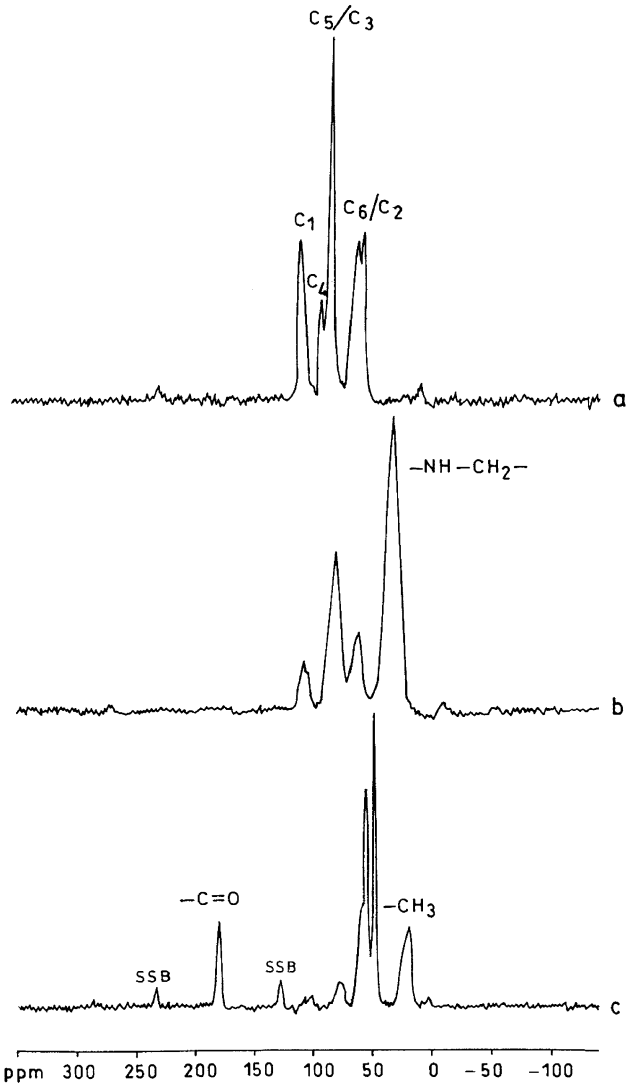

Fig. 4. CP-MAS ^{13}C NMR spectra of (a) chitosan, (b) C-*g*-PAN, (c) C-*g*-PMMA.

2001) at 33 ppm increased with increase in grafting in C-*g*-PAN. Broad multiple splitting of C1 centered at 102.8 ppm, C3/C5 at 76.8 ppm and C2/C6 at 60.76 were observed. The spectrum of C-*g*-PMMA showed –C=O and CH_3 signals at 180.5 and 18.9 ppm, respectively, along with signals at 106–100 and 77.8 ppm due to ring carbons. Nevertheless a dominance of PMMA intensity was ascribable to extended polymeric side chains. Based on spectral peak intensities we envisage slightly different mechanisms for these two copolymers. AN may undergo regular substitution at most of the amino radicals of chitosan, resulting in a frequent short PAN sidechain appendages on the chitosan backbone; whereas in the case of MMA the radical copolymerization may be taking place at a few amino radicals, with simultaneous elongation resulting in very long sidechains could be due to difference in reactive ratios of AN and MMA monomers.

3.1.4. Differential scanning calorimetry

In Fig. 5, thermograms of chitosan, C-*g*-PAN and C-*g*-PMMA show the endotherms with a peak temperature at around 150 °C, ascribable to the water holding capacity, which is possible through the –OH and unsubstituted, free $–NH_2$ groups of chitosan (Sato et al., 1997). Additionally, chitosan exhibits an exothermic peak temperature at 302 °C (Fig. 5a). From our earlier observations it is likely that a correlation between the decomposition peak temperature and degree of polymerization of chitosan is possible (Harish Prashanth, Kittur, & Tharanathan, 2002; Kittur, Harish Prashanth, Udaya Sankar, & Tharanathan, 2002). In graft copolymers, the decomposition peak temperature come down drastically from around 300 to 255–251 °C (Fig. 5b and c) because of free radical induced depolymerization of chitosan (Hsu, Don, & Chiu, 2002; Tharanathan & Harish Prashanth, 2001). Prior to exothermic peak initiation a small drift in the heat flow (indicated by an arrow in Fig. 5b and c) was noticed which may correspond to the amorphous chitosan region pendented with bulky side chains grafts. In C-*g*-PAN, with increase in decomposition peak temperature

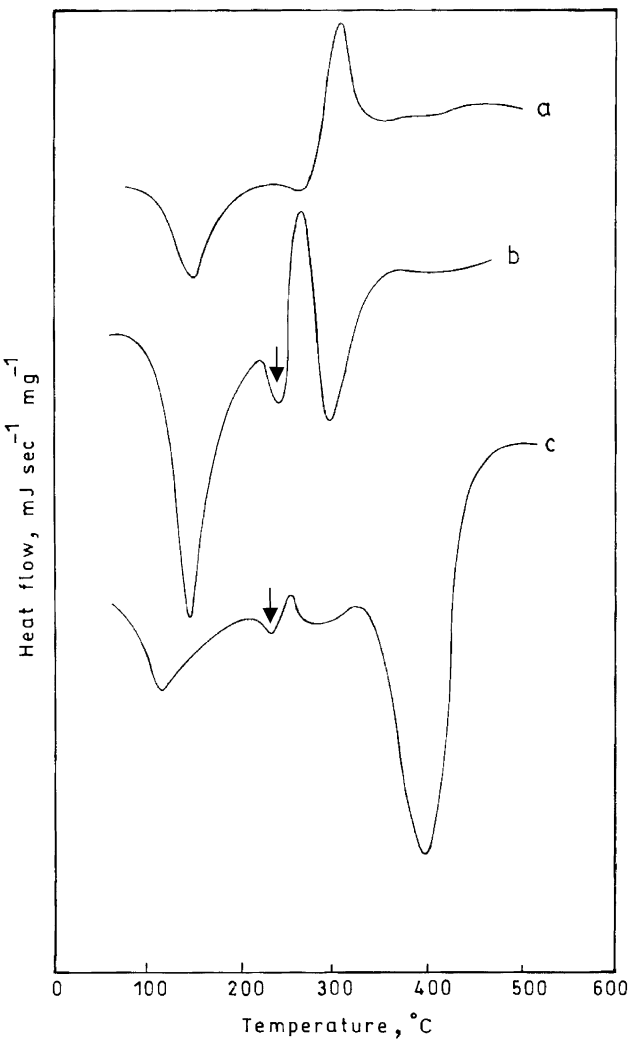

Fig. 5. DSC thermograms of (a) chitosan, (b) C-*g*-PAN and (c) C-*g*-PMMA.

the percent grafting showed a slight decreasing trend, viz. 145% C-*g*-PAN gave a decomposition peak temperature of 259.6 °C, whereas 170% graft copolymer gave 255 °C (Table 1). This may be attributed to the coinciding decomposition peak temperatures of PAN and chitosan. The C-*g*-PAN indeed showed only decomposition peak but not a melting peak, contrary to C-*g*-PMMA, which showed a the melting peak around 280–400 °C. As the percent grafting increased an increase in the melting peak temperature was observed, thus showing a good correlation ($r = 0.996$).

3.1.5. X-ray diffraction

The powder X-ray diffractograms of the graft copolymers showed distinct crystalline peaks compared to the native chitosan. The latter showed two major peaks at around 10 and 20° due to 020 and 110 reflections, respectively (figure not shown). The highly grafted C-*g*-PAN showed much variations in the peaks apart from the weak absorption at 21.04° and a strong absorption at 16.72°, which were due to PAN crystalline regions (Fig. 6). On the otherhand, the C-*g*-PMMA showed a single broad absorption at 13.5°, appeared to be due to extensive side chain coverage of PMMA, because the latter lacks complete stereoregularity due to its bulky methyl side groups (Ren & Tokura, 1994).

3.1.6. Scanning electron microscopy

Graft copolymerization considerably modifies chitosan morphology, and also its physical, chemical and biodegradable characteristics, which varies with respect to the nature of the synthetic side chains incorporated. SEM observations of native chitosan revealed its fibrous as well as flaky nature (Fig. 7a), wherein innumerable thin fibre strands deposited over the surface are visible. The fibrous nature of chitosan was totally modified in the grafted materials, wherein distinct morphological differences were discernible in their surface topography. C-*g*-PAN showed a soft porous structure (Fig. 7b), and C-*g*-PMMA showed the appearance of clustered irregular beads (Fig. 7c, Don, Hsu, & Chiu, 2001).

3.2. Residual monomer detection

The residual monomers if any, in the graft copolymers were extracted with water and subjected to an isocratic reverse phase-HPLC separation (Saroja et al., 2000). No detectable monomers were revealed even in C-*g*-PAN, although acrylonitrile is known for degradation if kept for long time, to acrylamide and acrylic acid (Zhang & Chen, 2001). Even a sample of C-*g*-PAN stored for 6 months did not show any residual monomers.

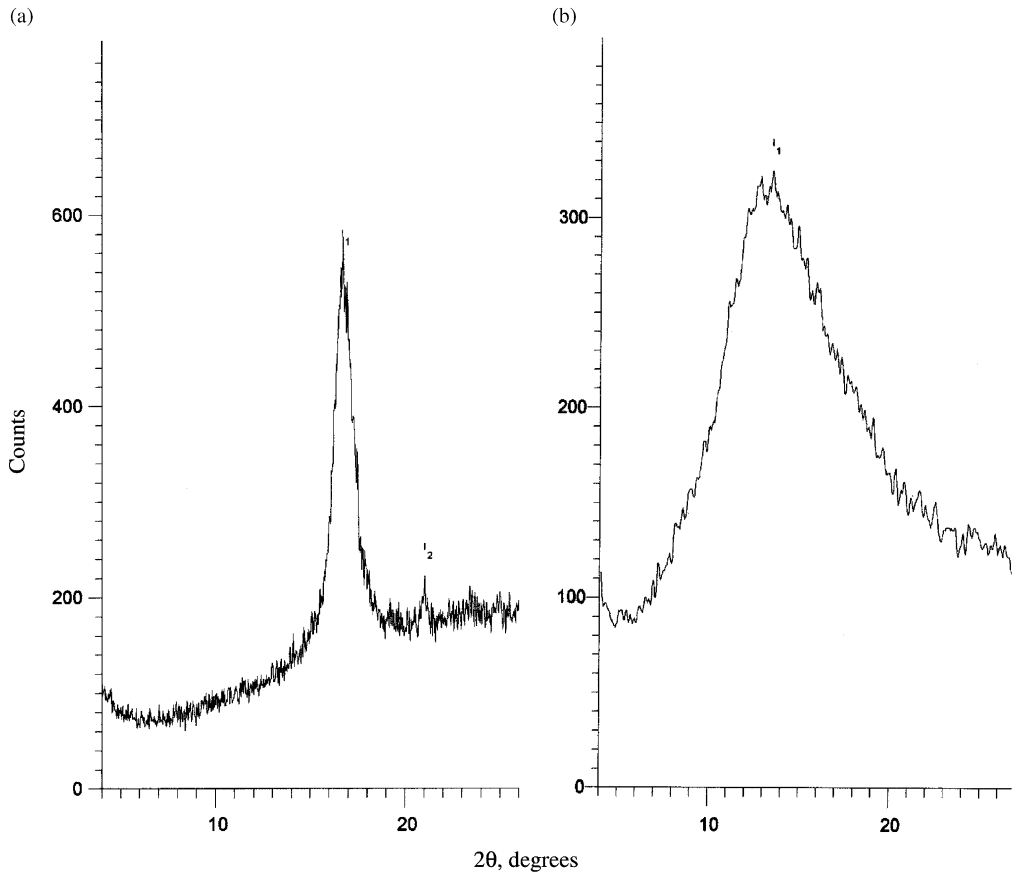

Fig. 6. X-ray diffraction patterns of (a) C-*g*-PAN, (b) C-*g*-PMMA.

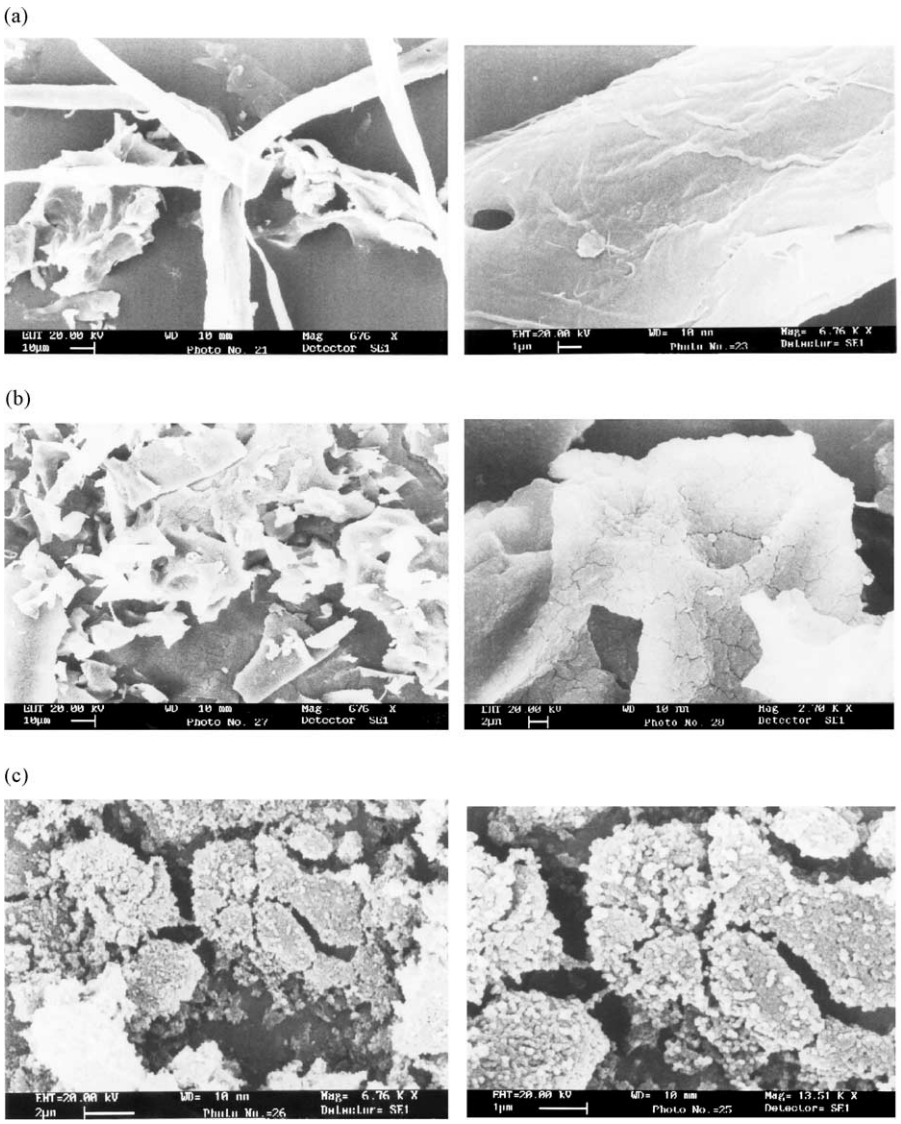

Fig. 7. SEM photographs—low and high resolution of (a) chitosan, (b) C-*g*-PAN, (c) C-*g*-PMMA.

3.3. Copolymer membrane

The dry solid copolymers on heat pressing between Teflon sheets gave semi-transparent, pale brown coloured thin films with C-*g*-PMMA, whereas the C-*g*-PAN film was too brittle for handling. The operating conditions to get C-*g*-PMMA film were 115 ± 2 °C for 20 min. The brittleness of the films can be overcome by incorporating plasticizers, and such films may possibly be used as biodegradable packaging materials.

4. Conclusions

Chitosan was graft copolymerized with synthetic monomers, viz. acrylonitrile and methylmethacrylate in the presence of potassium persulfate as a free radical initiator. Percent grafting with the latter was more, as evident from CP-MAS ^{13}C NMR. Thermograms of C-*g*-PMMA showed an increased peak temperature with increase in percent grafting. X-ray diffraction revealed changes in crystallinity of grafted copolymers. In scanning electron micrographs native chitosan appeared fibrous, whereas C-*g*-PAN and C-*g*-PMMA appeared flaky and beads-like, respectively. No residual monomers were found in the graft copolymers, even after storage for long periods. The graft copolymers could be thermopressed to thin membranous films.

References

Capitani, D., Angelis, A. A. D., Crescenzi, V., Masei, G., & Segree, A. L. (2001). NMR study of novel chitosan-based hydrogel. *Carbohydrate Polymers*, *45*, 245–252.

Chellapandian, M., & Krishanan, M. R. V. (1998). Chitosan-poly(glycydylmethacrylate)copolymer for immobilization of urease. *Process Biochemistry*, *33*, 595–600.

Chen, T., Kumar, G., Harris, M. T., Smith, P. J., & Payne, G. F. (2000). Enzymatic grafting of hexyloxyphenol onto chitosan to alter surface and rheological properties. *Biotechnology and Bioengineering*, *70*, 564–573.

Don, T. M., Hsu, S. C., & Chiu, W. Y. (2001). Structures and thermal properties of chitosan modified poly(methylmethacrylate). *Journal of Polymer Science: Polymer chemistry*, *39*, 1646–1655.

Harish Prashanth, K. V., Kittur, F. S., & Tharanathan, R. N. (2002). Solid state structure of chitosan prepared under different N-deacetylating conditions. *Carbohydrate Polymers*, *50*, 27–33.

Hsu, S. C., Don, T. M., & Chiu, W. Y. (2002). Free radical degradation of chitosan with potassium persulphate. *Polymer Degradation and Stability*, *75*, 73–83.

Kittur, F. S., Harish Prashanth, K. V., Udaya Sankar, K., & Tharanathan, R. N. (2002). Characterization of chitin, chitosan and their carboxymethyl derivatives by differential scanning calorimetry. *Carbohydrate Polymers*, *49*, 185–193.

Kojima, K., Yoshikuni, M., & Suzuki, T. (1979). Tributylborane initiated grafting of methylmethacrylate onto chitin. *Journal of Applied Polymer Science*, *24*, 1587–1593.

Ren, L., & Tokura, S. (1994). Structural aspects of poly(methylmethacrylate)-grafted β-chitin copolymers initiated by ceric salt. *Carbohydrate Polymers*, *23*, 19–25.

Rinaudo, M., Desbrieres, J., LeDung, P., ThuyBinh, P., & Dong, N. T. (2001). NMR investigation of chitosan derivatives formed by the reaction of chitosan with levulinic acid. *Carbohydrate Polymers*, *46*, 339–348.

Saroja, N., Gowda, L. R., & Tharanathan, R. N. (2000). Chromatographic determination of residual monomers in starch-g-polyacrylonitrile and starch-g-polyacrylate. *Chromatographia*, *51*, 345–348.

Sato, H., Tsuge, S., Ohtani, H., Aoi, K., Takasu, A., & Okada, M. (1997). Characterization of chitin-based polymer hybrids by temperature-programmed analytical pyrolysis techniques. 1. Chitosan-graft-poly(2-methyl-2-oxazoline)/poly(vinyl chlorides) blends. *Macromolecules*, *30*, 4030–4037.

Shanthi, C., & Panduranga Rao, K. (2001). Chitosan modified poly(-glycidylmethacrylate-butyl acrylate) copolymers in grafted bovine pericardial tissue—anticalcification properties. *Carbohydrate Polymers*, *44*, 123–131.

Tharanathan, R. N., & Harish Prashanth, K. V (2001). A process for preparation of low molecular weight chitosan. Indian patent, 433/DEL/01.

Yalpani, M., Marchessault, R. H., Morin, F. G., & Monasterios, C. J. (1991). Synthesis of poly(3-hydroxyalkanoate) (PHA) conjugates: PHA-carbohydrate and PHA-synthetic polymer conjugates. *Macromolecules*, *24*, 6046–6049.

Zhang, L. M., & Chen, D. Q. (2001). Grafting of 2-(dimethylamino)ethylmethacrylate onto potato starch using potassium permanganate/sulfuric acid initiation system. *Staerke*, *53*, 311–316.