BUTTERWORTH HEINEMANN

Polymer Vol. 36 No. 15, pp. 2915–2919, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0032-3861/95/$10.00+0.00

Differential scanning calorimetry investigation of phase transitions in water/chitosan systems

JoAnn Ratto* and Tatsuko Hatakeyama†
National Institute of Materials and Chemical Research, Tsukuba, Ibaraki 305, Japan

and Rita B. Blumstein
Polymer Program, University of Massachusetts Lowell, Lowell, MA 01854, USA

(Received 5 October 1994; revised 15 February 1995)

Transition temperatures of water/chitosan systems with water contents ranging from 8 to 300% (weight per cent of water per dry polymer weight) are measured by differential scanning calorimetry (d.s.c.). Upon heating, three kinds of phases, which vary with the water content, are observed for the system: cold crystallization, melting of freezable water, and a birefringent to isotropic phase which occurs for samples containing 44–190% water with a transition temperature that does not vary significantly with the water content. The glass transition temperature (T_g) of chitosan is observed at approximately 303 K for water contents ranging from 8 to 30%. From the d.s.c. data, a transition map for the water/chitosan system is compiled.

(Keywords: chitosan; water; d.s.c.)

INTRODUCTION

Chitin is a natural polysaccharide which forms the base for the hard outer integuments of crustaceans, insects and other invertebrates. It is prepared by the deacetylation of chitin (a naturally occurring linear polymer of $\beta(1 \rightarrow 4)$ poly-*N*-acetylglucosamine) in aqueous media and has the structure shown in *Figure 1*[1]. The applications of chitosan in the agricultural, medical, food and cosmetic industries are described elsewhere[1,2].

Many investigations have been reported[3–12] of water/polysaccharide systems containing the $\beta(1 \rightarrow 4)$ linkage and showing the presence of a mesophase at around room temperature with various water contents. The liquid crystal transition temperature is influenced by the water concentration. Various water species are observed and are classified as non-freezing, freezing bound and bulk-like water.

There have been few studies on the liquid crystallinity of chitosan[13–17]. Ogura *et al.* investigated liquid crystalline phases in concentrated solutions of chitosan, hydroxypropyl chitosan, and acetoxypropyl chitosan by light microscopy[13]. Sakurai *et al.* reported liquid crystal structures in chitosan films and fibres prepared from liquid crystalline solutions[14,15]. Recently, Terbojevich *et al.* performed light scattering measurements on chitosan with various degrees of deacetylation in acetic acid–sodium chloride solution and found cholesteric mesophases in these systems[16]. Rout *et al.* similarly found cholesteric solutions in site-selectively modified chitosans dissolved in organic solvents[17].

In this study, the effect of water concentration on the phase structure of water/chitosan samples is investigated. By compiling a phase transition plot for this system it is hoped that a fuller understanding of the role of polysaccharide mobility can be achieved.

EXPERIMENTAL

Sample preparation

Pronova B-MV chitosan glutamate, which during preparation was filtered through a 5 μm filter, was supplied by Protan Company. The chitosan glutamate was converted to neutral chitosan by dissolving it in distilled deionized water and stirring for several days. This solution was filtered, using Whatman number 2 filter paper, before 2 M sodium hydroxide was added dropwise to precipitate the chitosan. Once the pH of the solution reached 10.0, the chitosan and remaining solution were filtered through a sintered glass funnel. The chitosan was washed continuously with distilled water and dried under vacuum overnight at 313 K. The chitosan was then freeze-dried and a white powder was obtained in approximately 60% yield. The degree of deacetylation was determined to be 80% by infrared spectroscopy[18]. The molecular weights, determined by size exclusion chromatography and light scattering, were $M_n = (2.55 \pm 0.2) \times 10^5$ and $M_w = (3.5 \pm 0.2) \times 10^5$. The ion contents were measured using a Perkin-

---

*Present address: US Army Natick Research, Development and Engineering Center, Kansas Street, Natick, MA 01760, USA
†To whom correspondence should be addressed

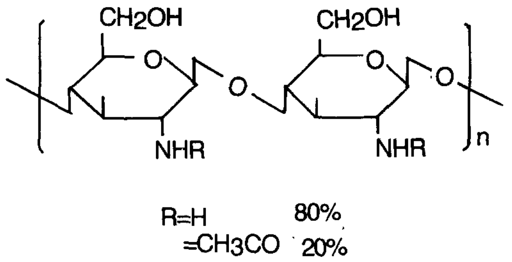

Figure 1 Chemical structure of chitosan

Elmer 6000 inductively coupled plasma atomic emission spectrometer and the following ions were found in the neutral chitosan: Mg^{2+}, Fe^{3+} and P^{5+} containing 2.0. 3.4 and 3.4 mg/100 g, respectively.

Differential scanning calorimetry

A Seiko DSC 200 fitted with a liquid nitrogen cooling accessory was used for all differential scanning calorimetry (d.s.c.) experiments. The sample vessels were first placed in the sample holder at room temperature and quench-cooled to 150 K. Measurements were performed upon heating and cooling over the temperature range 150–330 K at a rate of 10 K min^{-1}. Several heating and cooling cycles were performed to ensure reproducibility of the data.

Hermetically sealed aluminium d.s.c. sample vessels were used for all d.s.c. experiments and were boiled for several hours in distilled water to eliminate any possibility of aluminium hydroxide ($Al(OH)_3$) formation during the experiment. The chitosan was weighed (5 mg) in the d.s.c. vessel, excess distilled deionized water was added to the sample vessel and the water was allowed to evaporate until the desired water content was obtained. The sample vessels were then sealed and remained at room temperature for several days to allow the sample to equilibrate. The sample vessels were weighed again before the start of the experiment and reweighed afterwards to ensure that no weight loss had occurred during the experiment.

Following completion of the d.s.c. measurements, the sample vessels were punctured with tweezers and placed in an oven under reduced pressure at 380 K for 24 h to dry the samples. The dried samples were weighed immediately upon removal from the oven to obtain the dry weight of the chitosan.

The water content, W_c, is defined as:

$$W_c(\%) = (W_w/W_s) \times 100$$

where W_w and W_s are the weight of the water in the sample and the weight of the dry chitosan powder, respectively. In this study, the water content varied from 8 to 300% (w/w).

Optical microscopy

A Leitz Orthoplan Pol polarizing light microscope equipped with a Linkam THMS 600 temperature control unit (150–400 K, +1 K) was used at 293 K to examine the high-order structure of the water/chitosan samples.

RESULTS AND DISCUSSION

Representative d.s.c. cooling and heating curves for the water/chitosan systems with various water contents are shown in *Figures 2A* and *B*, respectively. *Figures 2C* and *D* display the heating data on an expanded scale for W_c values of 44%, 32% and 18%. Hysteresis is observed between the heating and cooling cycles and there is significant depression of the freezing temperature with $\Delta T = 15$–40 K. At water concentrations below 44%, the freezing or melting of water is not observed, and only non-freezable water is present. However, over the same W_c range, a step-like increase in the heat capacity is recorded on the heating cycles between 295 and 310 K. A broad exotherm is recorded on heating in the region of 235 K for those samples with a W_c range of 45–65%. At higher water concentrations the freezing and melting of freezable water is observed. The presence of several broad exotherms in the 56% sample indicates a broad distribution of water environments in the sample. Even at higher water contents, the cooling exotherms remain broad. The melting endotherms are also broad and structured. For the W_c range of 44–150%, the melting point is lower than 273 K. The area under the d.s.c. peak represents the change in enthalpy associated with the freezing or melting of freezable water, and is plotted as a function of water content in *Figures 3A* and *B*, respectively. The slope of the linear plot gives the differential heat associated with the freezable water and the intercept on the horizontal axis is the maximum amount of non-freezable water (W_{NFmax}) in the hydrated polymer. The following values were obtained: on the cooling cycle $\Delta H_{crystallization} = 306 \pm 34\,J\,g^{-1}$, $W_c = 47 \pm 6\%$; on the heating cycle $\Delta H_{fusion} = 336 \pm 30\,J\,g^{-1}$, $W_{NFmax} = 41 \pm 7\%$. The values of ΔH are not constant, but are close to that of bulk water ($333\,J\,g^{-1}$).

The neutral chitosan powder used in this study (see Experimental section) is highly crystalline and, under ambient humidity, contains only loosely bound water which can be removed by drying to 383–493 K[19]. Its X-ray diffraction pattern is similar to the Type I polymorph described by Samuels for neutral chitosan[20]. Water molecules associated with the crystalline regions are loosely bound between the chitosan chains along the [010] direction[21].

Bound water is associated with the amorphous regions of chitosan chains which undergo a glass transition for $W_c \leq 44\%$. The data presented in *Figures 2* and *3* indicate that a large fraction of the water present in this content range is non-freezing and is closely associated with the polysaccharide matrix. The glass transition region displays a strong enthalpy relaxation peak, as usually found in quench-cooled samples. With increasing W_c (> 45%), the presence of freezing bound water as well as non-freezing water is detected and the chitosan powder becomes a gel in which the polysaccharide forms an infinite network in an aqueous medium. In the gel, the chitosan chains are more mobile than in the solid state, making it very difficult to determine at what temperature general motion of the polysaccharide chains is established. (For $W_c = 56\%$ a T_g is observable on cooling in the vicinity of 179 K.) As W_c increases further, chitosan hydrogel is formed which contains non-freezing, freezing bound and bulk-like water, and the notion of the glass transition in the chitosan component disappears. The glass transition temperature of bulk-like water is generally found in the region of 130 K.

The exotherm at 235 K on the heating cycle is attributed to cold crystallization of mobile water in the hydrated polymer. Cold crystallization is observed in water/chitosan systems over a very narrow water content range, where only non-freezing and freezing bound water are present. As the temperature is raised from 150 K, a portion of the non-freezing water becomes mobile and by coming into contact with the solidified freezing bound water forms ice. Therefore, an exotherm is observed during the heating cycle. This ice melts in the region of 273 K with the other ice species present in the hydrated polymer network. This phenomenon has been observed in other hydrated polymer systems, where the proportion of non-freezing water which undergoes cold crystallization was quantified by measuring the mobile water n.m.r. relaxation times[22].

A small, fairly broad endotherm is observed on the heating cycle in the vicinity of 300 K for intermediate water contents ($W_c = 45$–190%). Polarizing microscopy indicates the presence of birefringence and a possible liquid crystalline structure, as illustrated in *Figure 4*. The enthalpy associated with this transition ranges from 0.8 to $2\,J\,g^{-1}$, with a maximum at $W_c = 125\%$. In the presence of small amounts of water, the mobility of chitosan chain segments is low and rearrangement of polymer chains is confined to the glass transition of the amorphous regions. However, within a certain W_c range, in this case 44–190%, the polysaccharide chain appears to attain sufficient mobility that additional molecular rearrangement can occur.

A phase transition map was compiled from the d.s.c. data and is presented in *Figure 5*. The glass transition temperature decreases with increasing W_c, suggesting that at low concentrations water plasticizes the chitosan matrix. The melting temperature of the freezable water increases with W_c up to 150% and then levels off. The

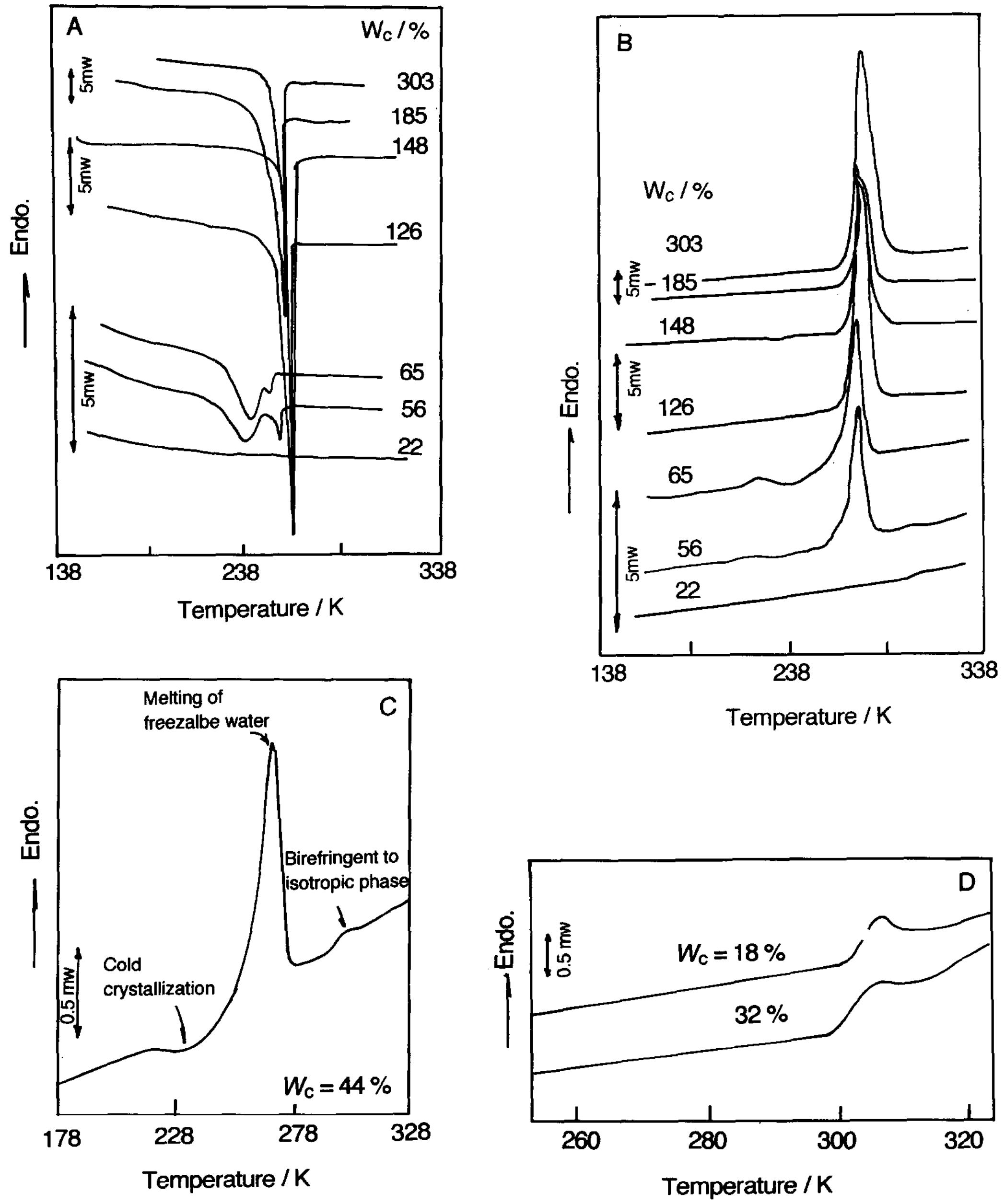

Figure 2 Representative d.s.c. cooling (A) and heating (B, C and D) curves as a function of water concentration, recorded at $10\,K\,min^{-1}$

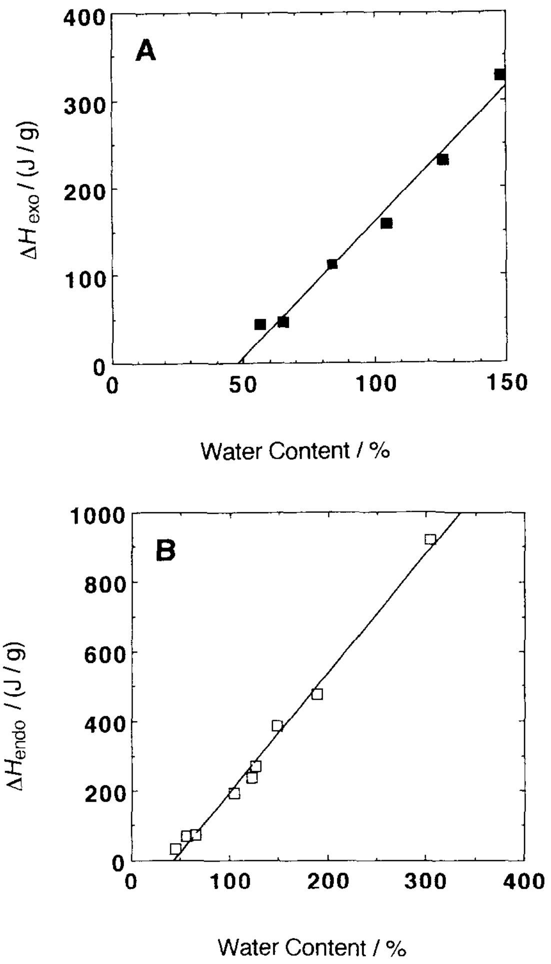

Figure 3 Total area of the d.s.c. peak per gram of dry chitosan associated with freezing (A) and melting (B) of freezable water. ΔH_{fusion} and W_{NF} are calculated from the slope of the line and the intercept on the horizontal axis, respectively

low melting temperature recorded below $W_c = 150\%$ may be caused by the formation of amorphous ice in this system. Similar behaviour has been noted in water/xanthan and water/sodium sulfate systems[3].

The nature of the birefringent phase and the weak endotherm observed in the vicinity of 303 K for intermediate water contents are difficult to interpret in the absence of further studies. Chitosan, like other polysaccharides, may form liquid crystalline solutions as well as gel phases[23]. As a weak polybase with pK_a 6.5, chitosan is readily soluble in aqueous acids[24]. In the present case, however, we are not dealing with solutions, since neutral chitosan (with the exception of some chitin-chitosans in the range of 40–60% deacetylation) is insoluble in pure water. The water-swollen amorphous regions are constrained by the crystalline regions. Formation of an anisotropic gel network may ensue upon melting of the frozen water. The endotherm observed in the vicinity of 303 K could possibly represent a gel–sol or liquid crystalline transition. Recently, the existence of free-standing mesomorphic gel with a reversible gel–liquid crystal sol transition below the liquid crystal–isotropic liquid transition has been reported for solutions of a site-selectively modified chitosan derivative[25]. In contrast to the liquid crystal–isotropic transition, the gel–sol transition temperature is rather insensitive to composition, just like the endotherm observed in this study.

CONCLUSION

Phase changes in water/chitosan systems have been studied by d.s.c. Cold crystallization, melting of water and glass transition of the hydrated chitosan are observed. An endotherm attributed to a birefringent to isotropic phase is observed for intermediate water contents. The nature and concentration of the water influence the mobility of the polysaccharide chains and the phase structure of this system.

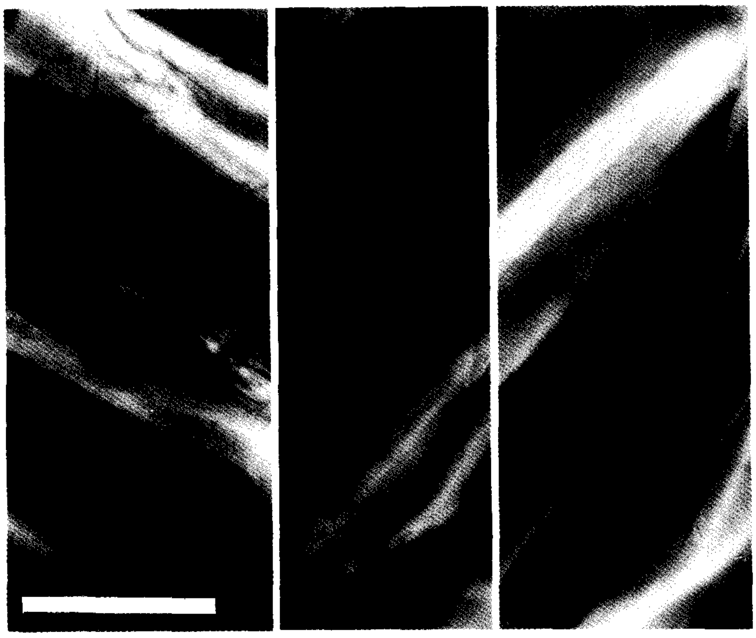

Figure 4 Structure in water/chitosan systems observed with a polarizing light microscope at room temperature. The bar represents a distance of 0.2 mm

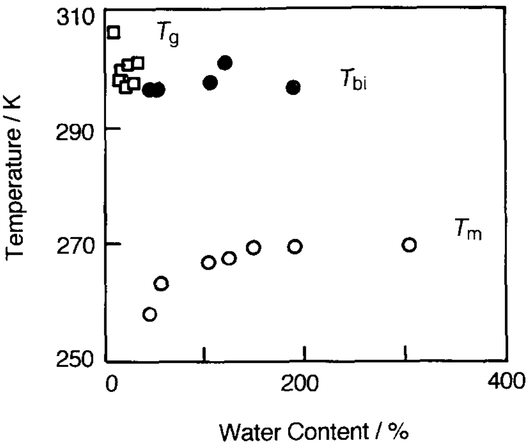

Figure 5 Phase transition map for water/chitosan system compiled from d.s.c. data. T_g, T_m and T_{bi} denote the glass transition, melting and birefringent phase temperatures, respectively

ACKNOWLEDGEMENTS

One of the authors (J.A.R.) gratefully acknowledges the National Science Foundation, The Japan Foundation Center for Global Partnership, the Japan International Science and Technology Exchange Center, and the Science and Technology Agency who sponsored her for the Summer Institute in Japan Program. The authors are grateful to Margaret Robertson and Neil Helbling for assisting with the ion content experiments, and to Rajesh Beri for the molecular weight determinations.

REFERENCES

1 Muzzarelli, R., Jeniaux, C. and Gooday, G. W. (Eds) 'Chitin in Nature and Technology', Plenum Press, New York, 1985
2 Skajak-Braek, J., Anthonson, T. and Stanford, P. (Eds) 'Chitin and Chitosan Sources: Chemistry, Biochemistry Physical Properties and Applications', Elsevier Applied Science, New York, 1989
3 Yoshida, H., Hatakeyama, T. and Hatakeyama, H. *Polymer* 1990, **31,** 693
4 Quinn, F. X., Hatakeyama, T., Yoshida, H., Takahashi, M. and Hatakeyama, H. *Polym. Gels Networks* 1993, **1,** 93
5 Nakamura, K., Hatakeyama, T. and Hatakeyama, H. *Polymer* 1981, **22,** 473
6 Nakamura, K., Hatakeyama, T. and Hatakeyama, H. *Polymer* 1983, **24,** 871
7 Hatakeyama, T., Nakamura, K., Yoshida, H. and Hatakeyama, H. *Thermochim. Acta* 1985, **88,** 223
8 Hatakeyama, T., Yoshida, H. and Hatakeyama, H. *Polymer* 1987, **28,** 1283
9 Nakamura, K., Hatakeyama, T. and Hatakeyama, H. in 'Wood and Cellulosics' (Ed. J. F. Kennedy), Ellis Horwood, Chichester, 1987, p. 97
10 Hatakeyama, T., Nakamura, K., Yoshida, H. and Hatakeyama, H. *Food Hydrocolloids* 1989, **3**(4), 301
11 Hatakeyama, T., Bahar, N. and Hatakeyama, H. *Sen-*i *Gakkaishi* 1991, **47**(8), 417
12 Nakamura, K., Hatakeyama, T. and Hatakeyama, H. *Polymer* 1991, **23**(4), 253
13 Ogura, K., Kanamoto, T., Sanno, T., Tanaka, K. and Iwakura, Y. 'Second International Chitin/Chitosan Conference', Tokyo, Japan, 1982
14 Sakurai, K., Miyata, M. and Takahashi, T. *Sen-*i *Gakkaishi,* 1990, **46**(2), 79
15 Sakurai, K. and Takahashi, T. *Sen-*i *Gakkaishi* 1988, **44**(3), 149
16 Terbojevich, M., Cosani, A., Conio, G., Marsano, E. and Bianchi, E. *Carbohydr. Res.* 1991, **209,** 251
17 Rout, D. K., Pulapura, S. K. and Gross, R. A. *Macromolecules* 1993, **26,** 5999
18 Domszy, J. G. and Roberts, G. A. F. *Makromol. Chem.* 1985 **186,** 1671
19 Ratto, J. Thesis, University of Massachusetts Lowell, 1993
20 Samuels, R. J. *J. Polym. Sci.* 1985, **18,** 1081
21 Ogawa, K., Hirano, S., Miyanashi, T., Yui, T. and Watanabe, T. *Macromolecules* 1984, **17,** 973
22 Smyth, G., Quinn, F. X. and McBrierty, V. *Macromolecules* 1988, **21,** 3198
23 Rinaudo, M. and Domard, A. 'Chitin and Chitosan: The Fourth International Conference on Chitin and Chitosan', Trondheim, 1988
24 Kurita, K. in 'Chitin in Nature and Technology' (Eds R. Muzzarelli, C. Jeuniaux and G. W. Godday), Plenum Press, New York, 1985
25 Rout, D. K., Pulapura, S. K. and Gross, R. A. *Macromolecules* 1993, **26,** 6007