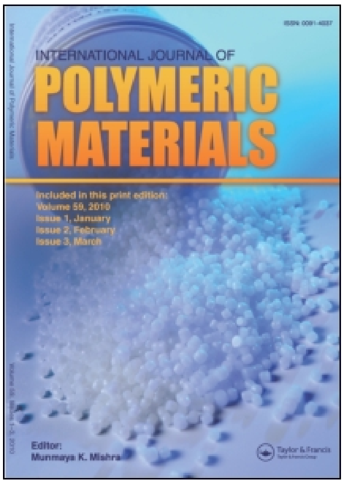
This article was downloaded by:
On: *19 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Polymeric Materials

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

Effects of additives and inert gas bubbling on the deacetylation of chitosan

Sérgio Paulo Campana-Filho[a]; Roberta Signini[a]
[a] Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brasil

Online publication date: 27 October 2010

To cite this Article Campana-Filho, Sérgio Paulo and Signini, Roberta(2002) 'Effects of additives and inert gas bubbling on the deacetylation of chitosan', International Journal of Polymeric Materials, 51: 8, 701 — 709
To link to this Article: DOI: 10.1080/714975830
URL: http://dx.doi.org/10.1080/714975830

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

International Journal of Polymeric Materials, 51:701 – 709, 2002

0091-4037/02 $12.00 + .00
DOI: 10.1080/00914030290046255

EFFECTS OF ADDITIVES AND INERT GAS BUBBLING ON THE DEACETYLATION OF CHITOSAN

Sérgio Paulo Campana-Filho and Roberta Signini
Instituto de Química de São Carlos-Universidade de São Paulo,
São Carlos/SP-Brasil

Commercial chitosan was purified, dissolved in 1.5% acetic acid and then submitted to deacetylation in aqueous sodium hydroxide solution at 110°C during 3h. Different conditions were employed to minimize the simultaneous occurrence of depolymerization, including the bubbling of nitrogen through the reaction medium and/or the addition of sodium borohydride or anthraquinone. Severe depolymerization occurred except when some measure was taken to minimize it. However more acetylated products resulted in these conditions. Adding anthraquinone and/or bubbling nitrogen through the reaction medium resulted in less degraded chitosans but the addition of sodium borohydride was not so effective. The more deacetylated and less degraded products were obtained when purified chitosan acetate was submitted to deacetylation in the presence of anthraquinone and simultaneous bubbling of nitrogen.

Keywords: chitosan, deacetylation, depolymerization, sodium borohydride, anthraquinone, nitrogen atmosphere

INTRODUCTION

Chitin, a cellulose-like linear polysaccharide, is one of the most abundant organic materials, surpassed only by cellulose in the amount annually produced by biosynthesis [1]. Chitosan, its principal derivative, is generally prepared by deacetylation of the parent chitin and, although many different procedures and conditions have been proposed [2 – 7], the most common practice involves the use of aqueous sodium hydroxide solution as the deacetylating medium. The extent of the deacetylation reaction as well as the characteristics of the deacetylated product depend on the alkali concentration, reaction time and temperature, and average size of the solid particles of chitin [2, 7]. Usually, the one-step alkaline deacetylation of chitin results in

---

Received 27 December 2000; in final form 31 December 2000.
The authors are greatful to FAPESP and CNPq for financial support.

Address correspondence to Sérgio Paulo Campana-Filho, Instituto de Química de São Carlos-Universidade de São Paulo, Avenida do trabalhador sancarlense, 400, 13566-590 São Carlos/SP-Brasil. E-mail: scampana@iqsc.sc.usp.br

deacetylated products to the extent of 75–85% but prolonging the reaction time does not enhance the reaction efficiency while favoring the simultaneous depolymerization process. The adoption of a multistage deacetylation procedure in which the alkali treatments are intercalated with washing and drying steps enhances the deacetylation extent but the deacetylated product is progressively more degraded the higher the number of deacetylation steps, principally at elevated reaction temperatures [8].

The preparation of fully deacetylated chitosan is attained after the execution of various consecutive deacetylation steps but the occurrence of severe depolymerization calls for the adoption of preventive measures [2, 9, 10]. The use of inert atmospheres and the addition of reducing agents and oxygen scavengers to the reaction medium are measures commonly taken for minimizing the concurrent depolymerization during the deacetylation reaction [2, 3]. In this work, the effects of sodium borohydride and anthraquinone, alone or accompanied by nitrogen bubbling, on the deacetylation of chitosan were investigated. For this purpose, the deacetylation procedure of Rinaudo *et al.* [10], involving the previous dissolution of purified chitosan in dilute acetic acid followed by pouring the resulting solution into the deacetylating medium, was adopted.

EXPERIMENTAL

Deacetylation Reactions

Low molecular weight chitosan (Fluka/BioChimika), identified as sample $[F]_B$, was employed in this work. Different purification procedures were employed to result in purified chitosan in the neutral form or in the salt form. Neutral chitosan (sample $[F_n]$) was obtained by dissolution of the commercial chitosan in 1% aqueous acetic acid, filtration, neutralization by adding concentrated ammonium hydroxide, washing the precipitate with water and drying it at ambient conditions. Chitosan acetate ($[F_{ac}]$) was obtained by dissolution of commercial chitosan in 1% aqueous acetic acid, addition of aqueous sodium acetate (0.2 mol/L), filtration and addition of absolute ethyl alcohol to promote the precipitation of purified chitosan acetate. Following the washing of the precipitate with water/ethyl alcohol it was dried at ambient conditions. Chitosan hydrochloride ($[F_{cl}]$) was prepared by dissolution in dilute hydrochloric acid (0.05 mol/L), addition of sodium chloride aqueous solution, filtration, addition of ethyl alcohol until the precipitation of chitosan hydrochloride occurred. Following the extensive washings with ethyl alcohol/water mixtures and with absolute ethyl alcohol, chitosan hydrochloride was dried at ambiente temperature.

In a typical deacetylation, 3.0 g of purified chitosan were suspended in 300 mL of 1.5% acetic acid aqueous solution and stirred until complete dissolution was achieved. The resulting solution was poured in 80 mL of

20% NaOH aqueous solution and kept at constant mechanical stirring in a glass reactor thermostatized at 110°C ± 2°C. The deacetylation reactions proceeded for 3 h and, to minimize the occurrence of depolymerization, some of them were carried out in the presence of sodium borohydride (0.1 g $NaBH_4$/1 g chitosan), anthraquinone (0.003 g/1 g chitosan) and/or nitrogen bubbling.

Chitosan Characterization

The average degrees of acetylation of purified chitosans were determined by ^{1}H nmr spectroscopy in D_2O/HCl (100 : 1 v/v) at 80°C, by using a Bruker 200 spectrometer. The intrinsic viscosities were determined by capillary viscometry in 0.3 mol/L acetic acid/0.2 mol/L sodium acetate buffer ($pH \cong 4.5$). The pure solvent and the chitosan solutions were previously filtered through 0.22 µm membranes and the measurements were carried out at 25°C ± 0.01°C. The AVS-350 viscometer and the AVS-20 automatic burette, both from Schott-Gerate, were used for these determinations. This system allowed the sequential dilution of the chitosan solution directly into the glass capillary ($\phi = 0.53$ mm), by adding the previously programmed volumes of the solvent. Intrinsic viscosities resulted by extrapolation of the Huggins equation [11] to infinite dilution and the corresponding viscosity average molecular weigths ($\overline{M_v}$) were calculated by applying the values of K and a determined in the same solvent and temperature [12]. Infrared spectra were obtained by using the KBr disc technique employing a BOMEM MB-102 FTIR spectrometer. X-Ray diffraction analysis were carried out in a Universal Diffractometer URD-6 (Veb Cal Zeissgena) employing CuKα radiation and crystallinity indexes were determined according to the method proposed for cellulose and applied to chitosan [13].

RESULTS AND DISCUSSION

Results reveal that the more deacetylated product was prepared when no additives were present in the deacetylation medium but a severe depolymerization was observed in this case (Tab. 1). The depolymerization was much less severe when some measure was taken to minimize it but the deacetylation was not so efficient in these cases, as may be observed by comparing the values of average deacetylation degree of chitosan $[F]_0$ and of the other deacetylated products (Tab. 1).

These results suggest that the presence of oxygen favors the occurrence of depolymerization during the deacetylation of chitosan as carried out in this work. In fact, the intrinsic viscosity of the parent chitosan purified in the neutral form, sample $[F_n]$, is 2.9 times greater than that of chitosan $[F]_0$, obtained after the deacetylation of $[F_n]$ in the absence of additives. On the other hand, the sample obtained from $[F_n]$ by bubbling nitrogen through the

TABLE 1 Average degrees of acetylation ($\overline{DA}$), intrinsic viscosities ([η]), viscosity average molecular weight ($\overline{M\nu}$) and crystallinity index (CI) of chitosans deacetylated in the presence of $NaBH_4$, anthraquinone and under nitrogen bubbling

Sample[a]	*Additive/bubbling gas*	(%)$\overline{DA}$[b]	[η] (*mL/g*)[b]	$\overline{M_\nu} \times 10^{4}$[c]	*CI* (%)	A_{1381}/A_{2920}
[F_n]	–	26.0	689	17	68	0.56
$[F]_0$	–	8.0 ± 1.5	236 ± 46	4	73	1.0
$[F]_{SB}$	$NaBH_4$	12.0 ± 2.5	456 ± 1	10	73	–
$[F]_{AT}$	anthraquinone	14.3 ± 0.9	501 ± 28	11	74	0.76
$[F]_N$	N_2	12.6 ± 4.6	502 ± 38	11	76	0.73
$[F]_{SBN}$	$NaBH_4/N_2$	13.6 ± 4.6	474 ± 20	10	74	–
$[F]_{ATN}$	anthraquinone/N_2	13.5 ± 0.4	545 ± 16	12	74	0.94

[a] [F] stands for commercial chitosan and subscripts 0, SB, AT and N identify chitosans deacetylated in the absence of additives, in the presence of sodium borohydride, of anthraquinone and by nitrogen bubbling, respectively.

[b] Values correspond to the average of at least two independent deacetylations.

[c] $\overline{M_\nu}$ values were calculated from [η] by applying the appropriate parameters [12].

deacetylating medium, chitosan $[F]_N$, gives an intrinsic viscosity 1.4 times smaller than that of chitosan [F_n]. Adding anthraquinone, an oxygen scavenger, to the reaction medium resulted in sample $[F]_{AT}$, whose characteristics are very similar to those of chitosan $[F]_N$, supporting the idea that the oxygen present in the reaction medium plays a role in the depolymerization process. Combining the nitrogen bubbling and the addition of anthraquinone to the deacetylating medium resulted in sample $[F]_{ATN}$, whose value of intrinsic viscosity is closer to that of the original chitosan but even in this case some depolymerization has occurred.

The addition of sodium borohydride, a reducing agent, also decreased the rate of depolymerization and, in this case too, the simultaneous bubbling of nitrogen was more effective than using the additive alone. However, it is noticeable that, even adding 30 times more sodium borohydride than anthraquinone, the prevention of depolymerization was not so efficient.

The effects of the deacetylation reaction on the structure of chitosan were monitored by infrared spectroscopy (Fig. 1). The changes due to N-deacetylation are usually seen in the interval $1680\,cm^{-1} – 1620\,cm^{-1}$, whose quantitative analysis is used to measure the extent of the deacetylation. The bands in this region are attributed to the vibrational modes of amide bands, whose intensity decrease as a consequence of the deacetylation reaction. However, as a consequence of the low average degree of acetylation and due to the relatively small differences among the samples concerning their DA, the changes in band intensities and shiftings are not very clearly observed. Nevertheless, the careful analysis of this region of the infrared spectrum allows the distinction of two sets of chitosans: the more acetylated (samples

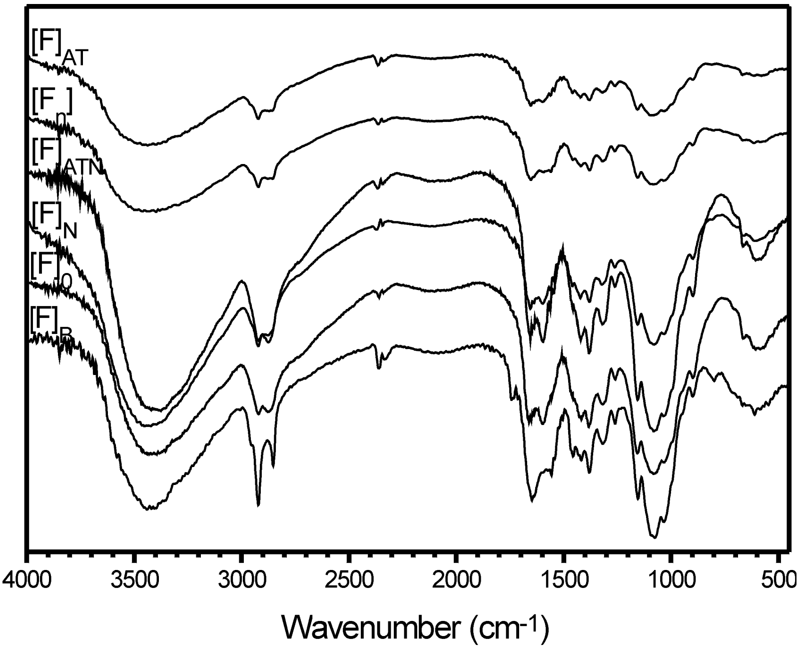


FIGURE 1 Infrared spectra of the commercial chitosan (sample $[F]_B$) and its purified neutral form (sample $[F_n]$), and deacetylated products $[F]_0$, $[F]_N$, $[F]_{AT}$, and $[F]_{ATN}$.

$[F]_B/[F_n]$ and $[F]_{AT}$) and the less acetylated (samples $[F]_0$, $[F]_N$ and $[F]_{ATN}$) chitosans.

The amide I and the amide II vibration modes occur as single bands at 1651 cm^{-1} and 1556 cm^{-1}, respectively. The first band is more intense and better resolved than the second one in all the spectra except in those corresponding to samples $[F]_0$, $[F]_N$ and $[F]_{ATN}$, the chitosans which have the lower average degrees of acetylation. Indeed, in the spectra of chitin and of highly acetylated chitosans the amide I vibration mode is split in two well resolved peaks at 1660 cm^{-1} and 1623 cm^{-1} while fully deacetylated chitosan (DA $< 5\%$) present only one band in the interval 1660 cm^{-1}–1580 cm^{-1} [14]. Another evidence to support the proposed classification of two groups of chitosans is found in the region of C—H stretching vibration. The band at 2960 cm^{-1}, attributed to CH_3 stretching due to the presence of acetamido groups, was observed as a small shoulder in the spectra of the more acetylated chitosans (samples $[F]_B$, $[F_n]$ and $[F]_{AT}$) but disappeared in the spectra of samples $[F]_0$, $[F]_N$ and $[F]_{ATN}$, the more deacetylated chitosans.

Other changes in the infrared spectra, although not taken as quantitative measures of the average degree of acetylation, reveal that different macromolecular arrangements occur as a consequence of the relative amount of amine and acetamido groups along the polymeric chains. The strong and large bands in the interval from 3600 cm^{-1} to 3000 cm^{-1}, which are due to O—H and N—H stretchings involved in inter and intramolecular hydrogen bonds [14], present different intensities and band widths, which have been taken as evidence for the occurrence of different types hydrogen bonds and diverse arrangements [14, 15]. The bands due to stretching vibration mode of C—H, relative to CH and CH_2, groups are well resolved in the spectrum of the commercial chitosan and centered in 2921 cm^{-1} and 2855 cm^{-1}. These bands are much less pronounced in the spectra of samples $[F_n]$ and $[F]_{AT}$ and that centered in 2855 cm^{-1} is shifted to higher frequency (2873 cm^{-1}) in the spectra of samples $[F]_0$, $[F]_N$ and $[F]_{ATN}$. The corresponding angular vibration modes occur in the interval 1500 cm^{-1} – 1200 cm^{-1}, the weak bands close to 1420 cm^{-1} and to 1380 cm^{-1} being assigned to CH_2 and CH bending, respectively. These bands are stronger in the spectrum of the parent chitosan and slightly shifted to higher frequency in the cases of samples $[F]_0$ and $[F]_{ATN}$. The set of bands corresponding to C—H vibration modes are claimed to be sensitive to the different macromolecular arrangements and that close to 1380 cm^{-1} should reflect the occurrence of conformational changes. In this sense, the absorbance ratio A_{1380}/A_{2920}, proposed as an index of crystallinity for cellulose [16], has been used to estimate the degree of order in chitin and chitosan [13] but it is not very well related to the crystallinity index determined by X-rays analysis in this work (Tab. 1), probably due to the errors associated with the low intensity of this band.

The X-rays analysis showed that the deacetylation of chitosan was in all cases accompanied by a small increase in the crystallinity index (Tab. 1), the diffractograms corresponding to the deacetylated products presenting a less symmetrical profile than that of the original chitosan. This is due to the appearance of a signal close to $2\theta = 23°$, also observed in the diffractogram of chitin [14, 15, 17], which is less pronounced in the case of sample $[F]_0$ (Fig. 2), the more deacetylated chitosan.

When suspended in dilute acetic acid, the chitosan chain acquires positive charges due to the protonation of its amino sites, resulting in its dissolution in the acid aqueous medium. Pouring this chitosan solution in the alkaline medium resulted in the immediate precipitation of the polymer. However, some authors [10] claim that, due to high swellability of this precipitate, the accessibility to acetamido sites on the polymer chains is not restricted. In this manner, these authors assume that in this condition chitosan is submitted to a homogeneous deacetylation.

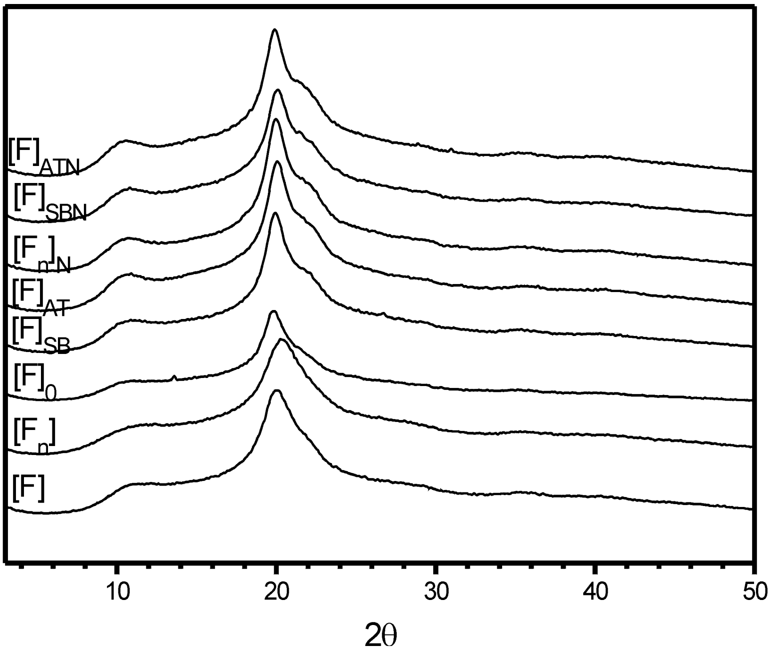


FIGURE 2 Diffractograms of sample [F], the purified forms, neutral chitosan (sample [F_n]) and deacetylated products corresponding to samples [F]$_0$, [F]$_N$, [F]$_{SB}$, [F]$_{AT}$, [F]$_{SBN}$, and [F]$_{ATN}$.

The X-rays analysis of chitosan acetate showed that this form of purified chitosan presented a different diffractogram as compared with those of commercial chitosan and its corresponding purified neutral form (Fig. 3), probably due to the presence of positive charges and its corresponding counter-ions in the case of the saline purified form.

To test the validity of assuming the chitosan deacetylation as a homogeneous reaction, chitosan acetate was submitted to deacetylation in the presence of anthraquinone and by bubbling nitrogen through the reaction medium, the best condition for preventing depolymerization found in this work. Carrying out this experiment twice resulted in deacetylated products whose characteristics ($[\eta] = 597 \pm 56\,\mathrm{mL/g}$ and $\overline{DA} = 9.6 \pm 3.0\%$) revealed that the reaction was more efficient and the rate depolymerization was lower than in any other case. These results suggest that the chitosan reactivity toward the deacetylation reaction, as carried out is this work, may depend on the accessibility to reactive sites. Preliminary experiments also showed that chitosan hydrochloride, whose X-rays analysis revealed that

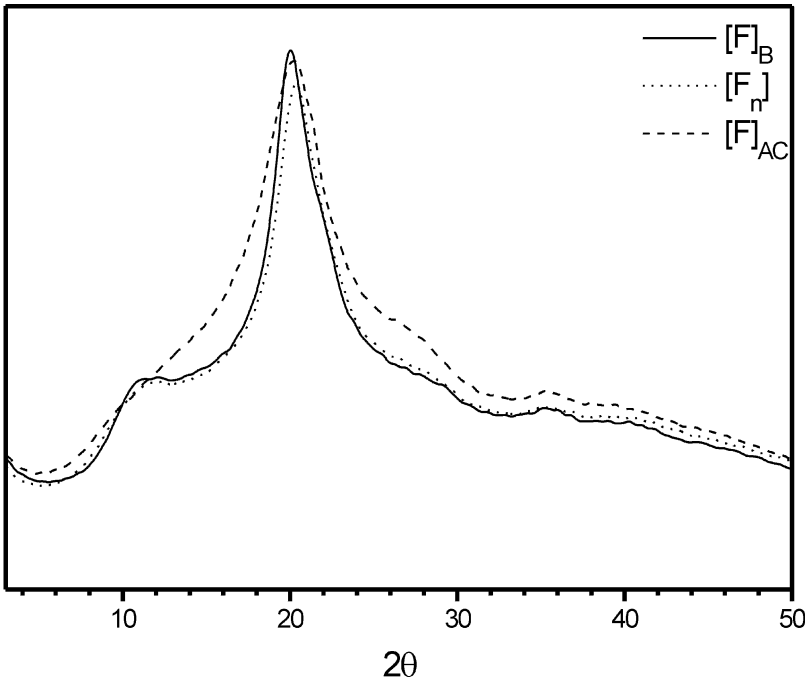


FIGURE 3 Diffractograms of the commercial chitosan (sample $[F]_B$) and purified forms: neutral chitosan(sample $[F_n]$) and chitosan acetate(sample $[F_{ac}]$).

this purified chitosan adopted an amorphous arrangement, presented a similar behavior toward the deacetylation reaction as that of chitosan acetate.

CONCLUSIONS

The use of additives, such as sodium borohydride and anthraquinone, and the bubbling of nitrogen through the reaction medium during the deacetylation of chitosan in aqueous sodium hydroxide, reduced the concomitant occurrence of depolymerization but the deacetylation itself was not as efficient in these cases as compared to the reaction carried out without any additive and in the presence of oxygen. Anthraquinone, even being added in a very small amount as compared to added sodium borohydride, was the most efficient additive and combining this with nitrogen bubbling resulted in less degraded chitosans. The deacetylation of chitosan seems to be a heterogeneous reaction as evaluated by comparing the reactivities of its salt and neutral purified forms.

REFERENCES

[1] Roberts, G. A. F., *Chitin Chemistry* (McMillan Press Ltd, London/UK, 1992), 1st edn., Chap. 1, pp. 2–3.

[2] Roberts, G. A. F., *Chitin Chemistry* (McMillan Press Ltd, London/UK, 1992), 1st edn., Chap. 2, pp. 64–77.

[3] Sashiwa, H., Saimoto, H., Shigemasa, Y. and Tokura, S. (1993). *Carbohydr. Res.*, **42**, 167.

[4] Horton, D. and Linebrack, D. R. (1965). *Meth. Carbohydr. Chem.*, **5**, 403.

[5] Madhavan, P. and Ramachadrannair, K. G. (1974). *Fish. Technol.*, **11**(1), 50.

[6] Kurita, K., Tomita, K., Tada, T., Ishii, S., Nishimura, S. and Shimoda, K. (1993). *J. Polym. Sci.: Part A: Polym. Chem.*, **31**(2), 485.

[7] Chang, K. L. B., Tsai, G., Lee, J. and Fu, W. (1997). *Carbohydr. Res.*, **303**(3), 327.

[8] Mima, S., Miya, M., Iwamoto, R. and Yoshikawa, S., *Chitin and Chitosan* (Hirano, S. and Tokura, S. (Eds.), The Japanese Society of Chitin and Chitosan, 1982), p. 21.

[9] Domard, A. and Rinaudo, M. (1983). *Int. J. of Biol. Macromol.*, **5**, 49.

[10] Dung, P., Milas, M., Rinaudo, M. and Desbrières, J. (1994). *Carbohydr. Polym.*, **24**, 209.

[11] Huggins, M. L. (1942). *J. Am. Chem. Soc.*, **64**, 1712.

[12] Rinaudo, M., Milas, M. and Dung, P. L. (1993). *Int. J. Biol. Macromol.*, **15**, 281.

[13] Struszczyk, H. (1987). *J. Appl. Polym. Sci.*, **33**, 177.

[14] Focher, B., Naggi, A., Torri, G., Cosani, A. and Terbojevich, M. (1992). *Carbohydr. Polym.*, **18**, 43.

[15] Focher, B., Belatrame, P. L., Naggi, A. and Torri, G. (1990). *Carbohydr. Polym.*, **12**, 405.

[16] Nelson, M. L. and O'Connor, R. T. (1964). *J. Appl. Polym. Sci.*, **8**, 1311.

[17] Signini, R. and Campana-Filho, S. P., *Proceedings of the III International Symposium on Natural Polymers and Composites (ISNaPol/2000)*, p. 131, São Pedro/SP-Brasil.