Properties and Adsorptive Capacity of Amino Acids Modified Chitosans for Copper Ion Removal

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Summary: This work presents the results of the modification of lateral groups of chitosan (2-amino-2-desoxy-β-D-glucose) by the reaction with different amino acids (glycine, L-lysine, -glutamic acid and L-isoleucine) under acid catalysis. The Cu^{2+} adsorption capacity of pure chitosan and of the chemically modified chitosans were also evaluated. The modification reaction favored the amide formation of the C-2 carbon of the glycoside ring under the adopted reaction conditions: reaction time and temperature and using sulfuric acid as a catalyst. The Cu^{2+} adsorption kinetics and equilibrium response using pure chitosan and the chemically modified chitosans as adsorbents showes that the adsorption capacity of equilibrium depended on the initial ion concentration. The response of each adsorbent gave good correlation with Langmuir's isotherm model. The following maximum adsorption capacity constants were obtained: 172.4 mg/g for chitosan and 69.9, 34.4, and 26.7 mg/g for modified chitosan with glycine, L-glutamic acid, and L-lysine, respectively. The adsorptive capacity seems to be dependent on the length and complexity of the added group.

Keywords: adsorption; amino acids; chitosan; copper; modified chitosan

Introduction

The increasing use of metals in industrial processes requires efficient methods to prevent pollution and promotes the recover and reutilization of these metals. The separation of metallic ions by adsorption has been focused, in recent years, in minimizing costs and decreasing environmental damages. An approach involves the use of biomaterials, which are easily available from nature or as wastes from processing natural products, and the development of better adsorbents by chemical modification of the existing biomaterials. Polysaccharides are biomaterials that have excellent physical and metal ion adsorption properties. Among them, chitosan, which is obtained from the deacetylation of chitin, has favored numerous studies of ion metallic uptake[1-5], because of the presence of both amine and hydroxyl groups that make it a very attractive material. In fact, it has been reported that

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chitosan is the biopolymer with the highest metallic ion chelation capacity ranging from 12 mg/g[6] to 92.4 mg/g[7]. Apparently, the amine groups in chitosan are the main responsible for the chelation of the metallic ions, as stated elsewhere[8-10]. Chitosan structure can be chemically modified seeking to improve its adsorptive capacity, since it has very reactive groups in each repetitive unit. There have been many chemical modifications of chitosan trying to incorporate in its structure different types of functional groups, cross-linking[11-14] or modifying its degree of deacetylation[15,16]. Some of the reported modifications towards better metal ion removal are carboxy-alkylation[3] and polyamination by using polyethylenimine[17]. Other approaches involve the modification of chitosan incorporating amino mercapto groups from tiirane, mercaptosuccinic acid, succinamide and piridoxal[18], as well as by preparing *n*-acetyl, nonanoyl[16], alkyl and benzylsulfonated chitosan derivatives[19], the mono and bis substitution of EDA and DTA in polystyrene[20,21] among others. On the other hand, amino acids have also been used as modifying agents of synthetic as well as natural polymers for the same purpose. Amino acids containing amino and one carboxylic group, and in some cases additional groups are present, which may act as potential chelation sites. Synthetic polymers modified with the introduction of amino acids are chloromethylated and chlorosulphonated polystyrene[22]; polymetacryloyl-glycine[23]; and glutamic acid grafted on polyurethane[24]. In the case of amino acid groups introduced to biomaterials, Muzzarelli [2] reported the reaction of α-ketoacids to introduce the corresponding amino acid group onto glucans for metal ion removal. Similar reactions were reported for cellulose. In another report, chitosan was reacted with dicyclohexylcarbodiimide in a standard addition reaction with cysteine and tryptophan, but in both cases the Cu^{2+} adsorption results were not as favorable as expected[18], mainly due to the low chelating potential for Cu^{2+} of the -SH group and to the complex structure of the attached group. In all cases, the linking unit was the amino group and the carboxylic group was on the free end of the introduced group. The reactions carried out to obtain the mentioned products generally use harsh conditions of pH, temperature, and not very environmentally benign reactants and solvents. Thus, in order to prepare modified chitosans favorable for copper ion removal, insoluble at the pH's commonly found in industrial wastewater, biodegradable and that could be obtained under more benign reaction conditions, this work was addressed to the syntheses of modified chitosans by reacting chitosan with different amino

acids such as glycine, L-lysine, L-isoleucine and L-glutamic acid, and to evaluate the Cu^{2+} ion adsorption capacity of pure chitosan and of the chemically modified chitosans.

Experimental Part

Chitosan with high molecular weight and degree of deacetylation (M_w = 600000, ~93% deacetylation from elemental analysis) was purchased from Fluka. The other reactants such as glycine, L-lysine, L-isoleucine, and L-glutamic acid were purchased from different laboratories (J. T. Baker, Beechman and Sigma-Aldrich, respectively). All reactants were used as received.

The chemically modified chitosan materials were obtained by the reaction of 4 g (0.025 moles) of chitosan dissolved in 750 mL of a 4 wt % aqueous solution of acetic acid with an equivalent double molar amount of the corresponding amino acid and 0.75 mL of concentrated H_2SO_4 as the catalysts[25]. All reactions of chitosan with the different amino acids were carried out at room temperature (~20°C) and pH = 2 in batch reactors with 1000 rpm mixing, with a mechanical stirrer, for 24 hours. The reaction products were precipitated in acetone to extract the modified polymers, were washed with distilled water three times and then were Soxhlet extracted with a 50 wt % mixture of methanol and acetone to remove residual reactants and solvents. The solids obtained were vacuum dried at 50 °C for three days. The used solvents were recovered by distillation.

Solubility tests at different pH's and temperatures were carried out using polar and non-polar solvents. FTIR Infrared Spectroscopy (Nicolet 540) was used to verify the introduction of new groups into the chitosan molecule during the reaction. High-vacuum Scanning Electron Microscopy (Phillips XL-30) was used to reveal special characteristics in the morphology of the pure and chemically modified chitosan and to give information on the elemental analysis of the samples.

The copper removal kinetics was evaluated at room temperature (~20°C) and pH = 5 in batch reactors using 100 mL of aqueous solutions containing different concentrations of the $CuCl_2 \bullet H_20$ salt. In every kinetic experiment, 0.5 g of chitosan and the corresponding modified chitosan were used as the adsorbent material and contacted with the copper solutions for a maximum period of 72 hours in a flask in a rotatory shaker at 100 rpm. Samples were taken from the reacting solution at different times. The copper concentration in the solution was

followed by Atomic Absorption (Varian Spectra 250 Plus) whereas the amount of Cu^{2+} adsorbed per gram of the adsorbent was calculated assuming that the amount of metallic ion that disappears from the solution is on the adsorbent material, since no considerable change in the pH of the solution that would favor the precipitation of the metal was observed during the adsorption process. Adsorption isotherms were constructed using the experimental equilibrium values reached with pure chitosan and with the modified chitosan materials.

Results and Discussion

From the solubility tests, it was observed that the chemically modified materials are insoluble in water, methanol, ethanol, acetone, ethyl-acetate, dicloroethane, cloroforms and hexane, as well as pure chitosan. They are also insoluble in 10 wt % aqueous solutions of hydrochloric acid, sulfuric acid, trichloroacetic acid, acetic acid and sodium hydroxide. Compared to pure chitosan, which is soluble at pH = 2 and partially soluble at pH = 4, the synthesized materials showed a higher chemical resistance to acidic condition since they are aparently insoluble at pH=2 and 4.

Fig. 1 shows the FTIR spectra of pure chitosan and of the chemically modified chitosans. There are two absorption bands associated with pure chitosan that may be modified by the chemical reaction between chitosan and the investigated amino acids. These bands are located at 1621 cm^{-1} and 1519 cm^{-1} and represent the N-H bond of associated amine groups. The absorption bands for the modified chitosans are different from those shown by pure chitosan, basically in the following spectral regions at about 2360 cm^{-1} at 1650 and 1540 cm^{-1}. The modified chitosans show absorption bands associated with the N-H bond of the amides, instead of the amine vibration shown by pure chitosan. These observations suggest that the reaction of chitosan with amino acids favors the amidation in the C-2 position of chitosan. Table 1 shows the proposed chemical structures of the modified chitosans.

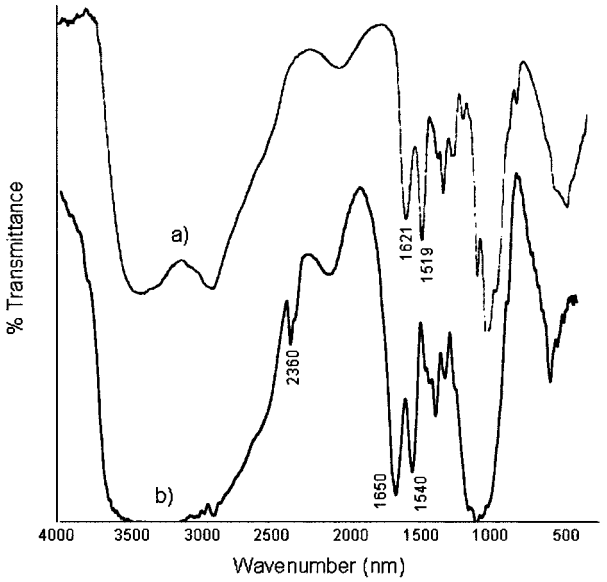
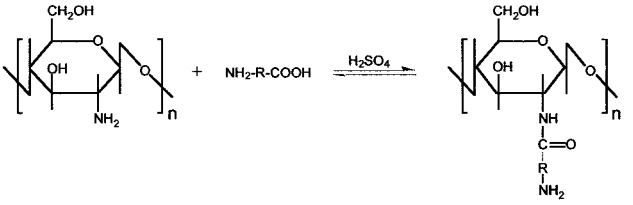


Fig. 1. FTIR spectra of (a) chitosan and (b) glycine-chitosan.

Table 1. Chemical structure of reactant and product of the modified chitosans with amino acids.



SEM images of the surface of pure chitosans and the chemically modified chitosans are shown in Figure 2. In all cases the surface characteristics of the modified chitosans are completely different from the surface of the original material. The products present a more continuous structure than chitosan and in the case of L-isoleucine-chitosan there are cumulus-like formations. Chitosan presents a more flaky structure with higher porosity.

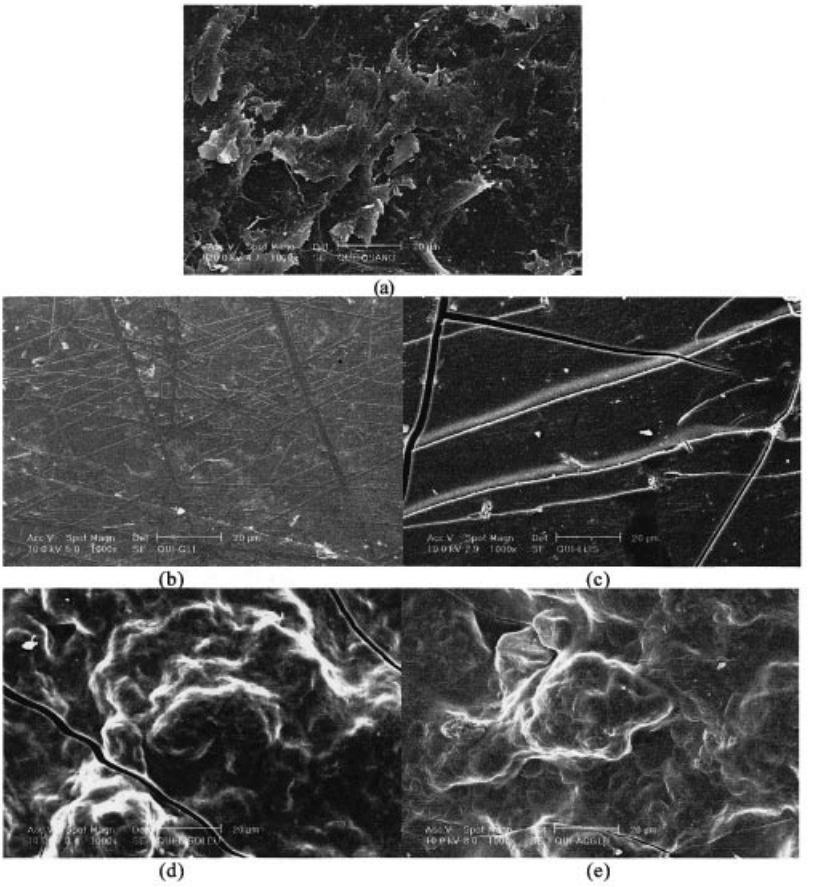


Fig. 2. Microscopy images of surface of (a) chitosan, (b) glycine-chitosan, (c) L-lysine-chitosan, (d) L-isoleucine-chitosan and (e) glutamic acid-chitosan.

An approximate degree of functionalization of each product was calculated by SEM elemental analysis. The approximate values were: glycine-chitosan, 88%, L-lysine-chitosan, 80%, L-isoleucine-chitosan 94% and glutamic-chitosan 99%.

Fig. 3 summarizes the Cu^{2+} adsorption kinetics by glycine-chitosan at different initial Cu^{2+} concentrations. Independently of the initial copper concentration, the Cu^{2+} ion kinetics removal show a non-linear behavior that reaches the equilibrium after approximately 30 h. It seems that the adsorption kinetics is highly dependent on the initial Cu^{2+} concentration, i.e., the Cu^{2+} ion equilibrium adsorption values increase with an increase in the initial concentration. All the other adsorbents studied here in this work have similar trends.

The solid lines in Fig. 3 represent the fit of the experimental data to a generalized-power law model equation of the form

$$\frac{dX}{dt} = k\,(X_{eq} - X) \qquad (1)$$

which in the integrated equation gives

$$X = X_{eq}(1 - e^{-kt}) \qquad (2)$$

In equation (1) and (2), X_{eq} is the equilibrium adsorption capacity of the material and k represents the "global" kinetic adsorption coefficient.

Generalized power-law equations have been successfully applied to describe the phosphorus release kinetics and substrate biodegradation kinetics carried out simultaneously by anaerobic microorganisms in a biofilm reactor[26] and the kinetics of copper uptake on fish scales[27] and chitosan[1]. As shown in Fig. 3, equation (2) can fit the experimental data quite well. The obtained parameters are presented in Table 2 that summarizes the fitted values, X_{eq} and k, for chitosan and modified chitosans.

As seen in Table 2 the k values for each adsorbent varies from 1.5 to 9.8 x 10^{-3} 1/h for chitosan, and from 2.3 to 7.3 x 10^{-3} for amino acid modified chitosans that in the average represents the behavior of the system. The little variations could due to the fact that the equation 1 is a generalized-power law equation that describes both the diffusion and adsorption processes.

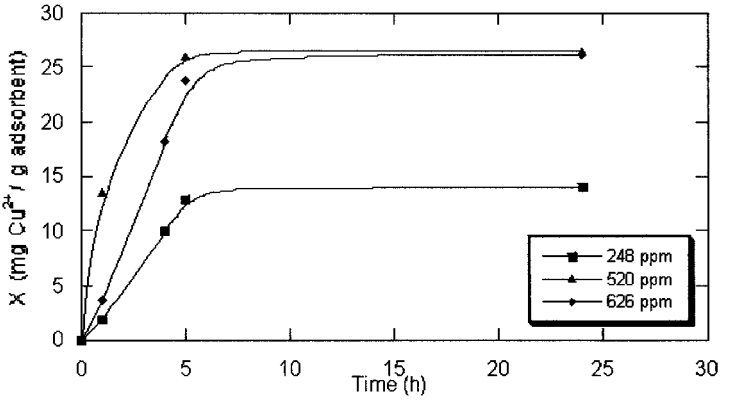


Figure 3. Cu^{2+} adsorption kinetics by glycine-chitosan at different initial Cu^{2+} concentrations.

Nevertheless, the results reported in Table 2 allow to set an order in the maximum copper adsorption capacity. The maximum Cu^{2+} adsorption capacity for the investigated adsorbents are in the order

$$X_{eq,Qui} > X_{eq,Qui-Gli} > X_{eq,AcGlu} > X_{eq,Qui-Lis}$$

Table 2. Kinetic parameters evaluated for Cu^{2+} adsorption by chitosan and modified chitosans.

Products	Cu^{2+} Initial Concentration	Parameters	
		X_{eq} (mg Cu^{2+}/g)	k x 10^3 (1/h)
Chitosan	62	6.0	2.4
	168	24.9	7.4
	268	40.4	1.4
	349	52.4	6.1
	390	52.6	9.8
	550	70.1	1.5
	626	101.1	5.9
Glycine-chitosan	248	14.8	3.9
	626	27.2	4.9
	887	30.4	2.8
L-lysine-chitosan	77	2.0	5.4
	331	7.8	4.1
	520	16.8	3.0
	626	27.1	7.3
	887	20.4	7.3
Glutamic acid-chitosan	86	5.8	2.7
	174	13.4	4.9
	265	13.3	2.3
	356	14.0	4.3
	450	12.9	2.3

This order can be related to the chain length of the incorporated amino acids since it increases from 2 to 6 carbons. The global kinetics adsorption coefficients of chitosan and modified chitosans do not show any correlation with either the initial concentration of the Cu^{2+} ion, or the chemical structure of the incorporated amino acid.

Fig. 4 shows a plot of X, the amount of Cu^{2+} adsorbed/g of adsorbent vs. $t^{1/2}$ for chitosan at different initial Cu^{2+} concentrations. For pure chitosan, there is a linear dependence of X on $t^{1/2}$ at low concentrations (< 400 ppm) as it was observed by other researchers[4]. This behavior suggests that the global Cu^{2+} ion adsorption is limited by a pore-diffusion process, at least at short times and low Cu^{2+} concentrations.

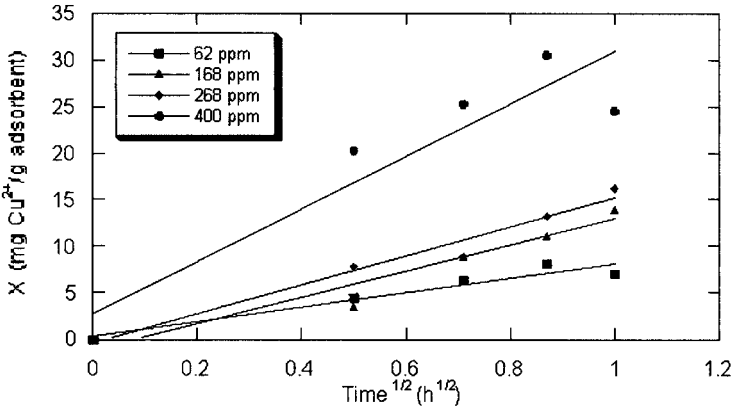


Figure 4. Diffuse adsorption kinetics by chitosan at different initial Cu^{2+} concentrations.

Fig. 5 presents the amount of Cu^{2+} adsorbed/g of adsorbent vs. $t^{1/2}$ for chitosan and the modified chitosans at 400 ppm initial Cu^{2+} concentration. As can be seen, at high concentrations (~ 400 ppm) there are deviations from linear behaviour that may suggest a more complex mechanism in the adsorption process.

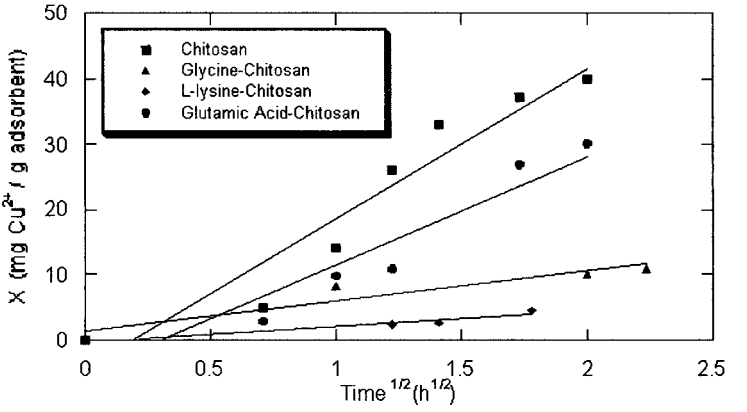


Figure 5. Diffuse adsorption kinetics by chitosan and amino acid modified chitosans, at 400 ppm Cu^{2+} initial concentration.

The isotherms that represent the Cu^{2+} adsorption by chitosan and the modified chitosans are shown in Fig. 6.

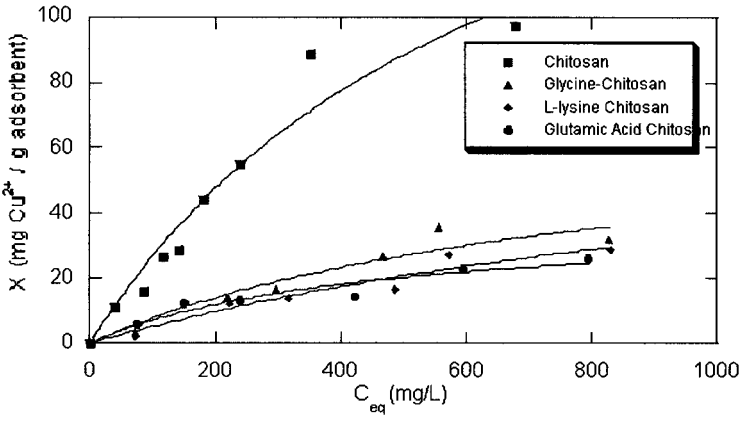


Figure 6. Cu2+ adsorption isotherms by chitosan and amino acid modified chitosans.

All adsorption isotherms were modeled by a Langmuir's equation

$$X = \frac{X_m k_s C_{eq}}{1 + k_s C_{eq}} \tag{3}$$

where X is the amount of Cu^{2+} mass adsorbed / adsorbent mass (mg Cu^{2+}/g), C_{eq} is the equilibrium concentration of Cu^{+2} in solution, X_m is the maximum amount required to cover the surface as a monolayer and k_s is equal to the inverse of concentration to half cover the monolayer and denotes the affinity between sorbent and sorbate. The values obtained for X_m and k_s are summarized in Table 3. As Fig. 6 shows the experimental data are well described by equation 3.

Table 3. Langmuir's isotherm model parameters for Cu^{2+} experimental adsorption for chitosan and modified chitosans.

Products	X_m (mg Cu^{2+}/g)	k_s x 10^3 (L/mg)	R^2
Chitosan	172.4	1.59	0.95
Glycine-Chitosan	58.8	0.69	0.98
L-lysine-Chitosan	26.7	0.98	0.97
Glutamic Acid-Chitosan	34.4	2.82	0.94

With respect to pure chitosan, the X_m values of the modified chitosans are lowered up to a maximum of 85%, as shown by glutamic acid-chitosan. The larger the amino acid molecule, the larger the reduction in X_m. The Langmuir's parameter, X_m, shows that the maximum adsorption capacity follows this order:

$$X_{m,Qui} > X_{m,Qui-Gli} > X_{m,Qui-AcGlu} > X_{m,Qui-Llis}$$

which is related to the length of the amino acid chain. This confirms what was observed in the kinetics studies.

Comparing the modified products among themselves, there seems to be a relation between the linearity and the degree of the functional group added, and the adsorption capacity of the carboxylic and amino groups.

Conclusions

Pure chitosan can be reacted with amino acids such as glycine, L-lysine, L-isoleucine and glutamic acid to produce chemically modified chitosans. Amide groups are preferentially formed on the C-2 carbon of the chitosan using the acid catalyzed reaction.

The obtained products are apparently insoluble in acidic solutions at pH=2 and 4 and have different morphology from pure chitosan.

The modified chitosans can adsorb Cu^{2+} ions from aqueous solutions at pH 5-6 but their adsorption capacity is lower than that of pure chitosan. The adsorption process for all products is well represented by Langmuir's isotherm model, and the removal kinetics fits a general power law equation. For chitosan and modified chitosans there seems to be a pore diffusion limiting step, specially at short times and low concentrations (< 400 ppm). The Cu^{2+} ion adsorption capacity by modified chitosans depends on the type and length of amino acid used and on the substitution degree[15]:

$$X_{m,Qui} > X_{m,Qui-Gli} > X_{m,Qui-AcGlu} > X_{m,Qui-Llis}$$

Additionally the adsorption values seem to follow the following order:

$$X_{\text{of neutral non-polarAA}} > X_{\text{of acid AA}} > X_{\text{of basic AA}}$$

The adsorption values measured and reported in this work well agree with those of other modified synthetic and natural polymers studied and reported elsewhere.

Acknowledgments

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[1] G. McKay, H. Blair, A. Findon, in: "*Chitin in Nature and Technology*", R. Rochetti, RA.., Muzzarelli, J. Jeuniaux, G.W. Gooday, Eds., Plenum Press, U.S.A. **1986,** pp. 559-565.
[2] R. A. Muzarelli, in: "*Chitin in nature and technology*", Proceedings from the 3rd Intl. Conf. on Chitin & Chitosan, Serriga, Italy, April 1-4, Plenum Press. U.S.A. **1986.**
[3] R. A Muzzarelli, in: "*Chitin-Chitosan: Sources, chemistry; biochemistry, physical properties & application*", Proceedings from the 4th Intl. Conf. on Chitin & Chitosan, Trondheim, Norway, Aug. 22-24, 1988, Elsevier Appl. Sci., U.S.A. **1989**. pp. 87-99.
[4] T. C. Yang, R. R. Zall, *Ind. Eng. Chem. Prod. Res. Dev.* **1984**, *23*, 168-172.
[5] K. A Almas,. A. Baradajan *et al,* in: "*Chitin in nature and technology*", Proceedings from the 3rd Intl. Conf. on Chitin & Chitosan,. Serriga, Italy, April 1-4, Plenum Press, U.SA. **1986.**
[6] K. Kurita, T. Sannan, Y. Iwakura, *J. Appl. Polym. Sci.* **1979**, *23*, 511-515.
[7] C. Ni, Y. Xu, *J. Appl. Polym. Sci.* **1996**, *59*, 499-504.
[8] Y. Kawamura, M. Mitsuhashi, H. Tanibe, *Ind. Eng. Chem. Res.* **1993**, *32*, 386-391.
[9] *"Chitin-chitosan: Sources, chemistry; biochemistry, physical properties & applications"*, Proceedings from the 4th Intl. Conf. on Chitin & Chitosan. Trondheim, Norway Aug 22-24, 1988. Elsevier Appl. Sci., U.S.A. 1989.
[10] *"Chitin in nature and technology"*, Proceedings from the 3th Intl. Conf. on Chitin & Chitosan. Serriga, Italy April 1-4. Plenum Press. E.U. 1986.
[11] R. H. F. Beck, M.G. Fitton, in: "*Handbook of Polymer Synthesis. Part B*", H.R. Kricheldorf, U.S.A. 1992, p. 1734.
[12] K. Kurita *et al., J. Appl. Polym. Sci.* **1986**, *31*, 1951-1954.
[13] T. Y. Hsien, G. L Rorrer,. *Ind. Eng. Chem. Res.* **1997**, *36*, 3631-3638.
[14] K.I.Draget, K.M.Varum, O. Smidsrod, in: "*Advances in Chitin and Chitosan*", C.J, Brine,. P.A. Sandford, J.P. Zikakis, Eds., Elsevier Science Publishers, U.S.A. **1992.** pp 685 –ff.
[15] K. Kurita, T. Sannan, Y. Iwakura, *J. Appl. Polym. Sci.* **1979**, *23*, 511-515.
[16] K. Kurita, Y. Koyama, S. Chikaoka, *Polym. J.* **1988**, *20*, 1083-1089.
[17] Y. Kawamura, M. Mitsuhashi, H. Tanibe, *Ind. Eng. Chem. Res.* **1993**, *32*, 386-391.
[18] C.L. Lasko, B.M. Pesic, D.J. Oliver, *J. Appl. Polym. Sci.* **1993**, *48*, 1565-1570.
[19] M. Weltrowski, B. Martel, M. Morcellet, *J. Appl. Polym. Sci.* **1996**, *59*, 647-654.
[20] M. B. Shambhu, M.C. Theodorakis, *J. Polym. Sci.* **1977**, *15*, 525-531.
[21] A. W. Trochimczuk, *Eur. Polym. J.* **1998**, *34*, 1657-1662.
[22] M.A. Petit, J. Jozefonvicz, *J. Appl. Polym. Sci.* **1977**, *21*, 2589-2596.
[23] S. Masuda, T. Miyahara, M. Tanaka, *J. Polym. Sci. Part A: Polym. Chem.* **1999**, 37, 1303-1309.
[24] M. Z. C. Hu, M. Reeves, *AIChE J.* **1999**, 45,11, p. 2333-ff.
[25] M. Yalpani, *" Polysaccharides. Synthesis, modifications and structure/property relations"*. Elsevier U.S.A. 1988, pp.1-44.
[26] F.A. Ruiz-Treviño, S. González-Martínez, *et al*, *Water Sci. Technol.*, **1992**, *36*, 813-820.
[27] J.F. Villanueva-Espinosa, M. Hernández-Esparza, F.A. Ruiz-Treviño, *Ind. Eng. Chem. Res.* **2001**, 16, 35/63-35/69.