Viscous and Viscoelastic Properties of **Chitosan Solutions and Gels**

Propriedades Viscosas e Viscoelásticas de Soluções e Géis de Quitosana

AUTORES

AUTHORS

Marco Antonio TORRES

PhD Candidate of State University of Campinas Faculdade de Engenharia Química, Universidade Estadual de Campinas, CP 6066 Barão Geraldo, Campinas, CEP 13083-852, Brasil Telephone: 55-19-3788 3893 FAX: 55-19-3788 3922 E-mail: marco@feg.unicamp.br

Marisa Masumi BEPPU

Cesar Costapinto SANTANA Professors of State University of Campinas

Eduardo José ARRUDA

Professor of Don Bosco Catholic University

All experiments were executed in the Bioseparation Laboratory and the Rheology Laboratory. School of Chemical Engineering and Mechanical Engineering. State University of Campinas, Campinas, São Paulo, Brazil.



Chitosan; Gels; Cross-linking; Response Surface; Viscoelasticity.

> Quitosana: Géis: Reticulação: Superfície de Resposta: Viscoelasticidade.

SUMMARY

The biopolymer chitosan is widely used in many fields, such as food processing technology, biotechnology, biomaterials, downstream processes and water purification. Along with the remarkable increase in chitosan applications has grown the need to process chitosan on a larger scale. The main goal of this work was the rheological characterization of chitosan samples previously dissolved in acetic acid solution and later cross-linked with glutaraldehyde. The samples were analysed after being submitted to changes in the temperature 10°C, 30°C and 50°C and concentrations 0.025 g.mL⁻¹, 0.03 g.mL⁻¹ and 0.035 g.mL⁻¹. The Ostwald-de Waele model and the Haake rheometer, operating under steady or oscillatory conditions were used to determine its viscous and viscoelastic properties. The results suggested that the thixotropic behaviour of the samples was influenced by the variables of temperature and concentration. The viscoelastic properties represented by the loss modulus, storage modulus and complex viscosity were dependent on the chemical composition of the samples.

RESUMO

A quitosana é um biopolímero que vem sendo amplamente utilizado em diversas áreas, como na tecnologia de processamento de alimentos, na biotecnologia, como biomaterial, em processos de recuperação e purificação, na purificação de água. Devido as notáveis aplicações da quitosana, cresce a necessidade de processar a quitosana em grande escala. Esse trabalho tem como objetivo principal à caracterização reológica de amostras de quitosana inicialmente solubilizadas em solução acética e posteriormente reticuladas com glutaraldeído. As amostras foram analisadas após variações de temperatura 10°C, 30°C e 50°C e de concentrações 0,025 g.mL⁻¹, 0,03 g.mL⁻¹ e 0,035 g.mL⁻¹. Para a determinação de suas propriedades viscosas e viscoelásticas foi utilizado o modelo de Ostwald-de Waele e um reômetro Haake operando em condições constantes e oscilatórias. Os resultados mostraram que o comportamento tixotrópico apresentado pelas amostras é influenciado pelas variáveis temperatura e concentração. A propriedade viscoelástica representada pelos parâmetros módulo de dissipação, módulo de rigidez e viscosidade complexa são dependentes da composição química das amostras.

1. INTRODUCTION

The polysaccharide chitin is commonly found in nature, present in many organisms such as fungi, seaweed, protozoa and arthropods (ROBERTS, 1992; TANGPASUTHADOL & PONGCHAISIRIKUL, 2003; LEE et al., 2001). Chitosan, a derivative of chitin, is of remarkable economic interest due to its functional versatility, with potential applications in the medical and pharmaceutical fields (HUANG et al., 2000; CHIRACHANCHAI et al., 2001; MUSLIM et al., 2001).

Chitosan is produced by the deacetylation of chitin to different degrees, and is formed from the β -(1 \rightarrow 4)-linked 2-amino-2-deoxy-D-glucopyranose (GlcN, D-unit) and 2acetamido-2-deoxy-D-glucopyranose (GlcAc, A-unit) structures with amino groups and primary and secondary hydroxyl groups. The presence of free amino groups in chitosan is responsible for its polycationic nature in acidic solutions.

An important advantage of chitosan is the possibility of chemical modifications in its structure by agents able to link to amino and hydroxyl groups (MONTEIRO & AIROLDI, 1999). MUZZARELLI (1977) studied the mechanism of cross-linking of chitosan with a bi-functional glutaraldehyde agent. The crosslinking reaction occurs between primary amino groups and aldehyde groups, resulting in the formation of Schiff bases. This complex reaction mechanism modifies the structure and functionality of chitosan, improving its chemical resistance.

DESBRIÈRES (1996) investigated the rheological properties of chitosan solutions and hydrogel systems with intermolecular association. The term "gel" can be used in different situations. Based on the studies of MORRIS (1990) and STEFFE (1996) on typical hydrocolloid materials, systems characterized by specific mechanical properties in which the storage modulus G'(w) presents a plateau with frequency (w) and the loss modulus G''(w) is smaller than G'(w), can be considered as gels. Therefore the complex viscosity (η^*) is almost inversely proportional to frequency. In concentrated solutions, at low frequency, the viscous response G''(w) predominates, and the response approximates that of a dilute solution. At high frequency, the elastic response predominates, and the response approximates that of gels. The complex viscosity decreases steeply with increasing frequency (w). At the opposite extreme, i.e., dilute solutions, the viscous response always predominates over the elastic response and the complex viscosity (η^*) approaches that of the dynamic viscosity (η'). The dynamic viscosity, measured with small deformation and dilute solutions, is in close quantitative agreement with the shear viscosity (η) , measured with large deformation. This behaviour is frequencyindependent (Newtonian response).

DRAGET (1996) studied the reaction of covalent glutaraldehyde and chitosan with different chemical compositions. SMIDSROD (2002), performed a study on branching chitosan. However, the mechanism and utility of modifying chitosan with hydrophobic branches to control the solubility properties, was not adequately demonstrated. The rheological characterization of hydrophobised chitosan derivatives is useful for many applications, involving different materials such as biologically active polymers, materials for immobilizing proteins, support materials and hydrophobic chromatography. In spite of these applications, most studies consider chitosan solutions rather than its gels. This work was also concerned with the effects of the chemical treatment of chitosan solutions with glutaraldehyde on their rheological behavious.

2. EXPERIMENTAL

2.1 Materials

Chitosan (Sigma, USA) with a minimum of 85 % of deacetylation (extracted from crab shells), was used. Nuclear, Brazil provided a 25 % (v/v) aqueous solution of glutaraldehyde.

Concentrated solutions were prepared by dissolving 2.5 % chitosan (mass) in an acetic acid solution containing 3 % (v/v) of acetic acid in Milli-Q water (water purified in an ion exchange cartridge). Homogeneous cross-linking was carried out in chitosan solutions by adding a 0.25 % (v/v) solution of glutaraldehyde in acetic acid and maintaining under continuous agitation for 1 hour. The time and concentration were previously determined to promote a complete reaction, avoiding the presence of unreacted glutaraldehyde in the gel. Bubbles of air were eliminated from the system by keeping the solution at 4°C for 2 hours. The gel formed was rheologically tested after 3 hours. Chitosan solutions and 0.025 g.mL⁻¹, 0.03 g.mL⁻¹ and 0.035 g.mL⁻¹ gels were tested at several temperatures: 10°C, 30°C and 50°C.

The samples were divided into two groups depending on the treatment: pristine chitosan solutions and gels. The molecular mass of chitosan was around 65 kDa, as determined by the viscosimetric method (BEPPU & SANTANA, 1999).

2.2 Rheometer

A plate-plate rheometer (Haake, model CV 20) having a plate diameter of 30 mm and a gap of 1 mm was used for the dynamic experiments. A rheometer capable of operating under steady or oscillatory conditions was used. With measurements made under steady conditions, the properties described are representative of the viscous behaviour of the samples, through values for shear stress (τ), shear rate (γ) and shear viscosity (η). The response (a), consistency index, and (n), the flow behaviour index, are related in the Ostwald-de Waele (or Power law) model as shown in Equation 1 and are typical parameters of pure viscous flow (SCHRAMM, 1994).

$$\tau = a (\gamma)^n \tag{1}$$

In the oscillatory measurements, the properties described are representative of the viscoelastic behaviour of the samples, through values for storage modulus (G'), loss modulus (G') and complex viscosity (η^*). For the oscillatory shear dynamic measurements, oscillatory external torque was applied to the

system. The material was submitted to a sinusoidal shear-strain. Equations 2 and 3 describe the responses to deformation and stress.

$$\gamma(t) = \gamma_0 \sin(wt) \tag{2}$$

$$\tau(t) = G^* \gamma_0 \cos(wt + \delta) \tag{3}$$

Equations 2 and 3 were used to calculate the shear storage modulus (G'), described by Equation 4, and the shear loss modulus (G"), described by Equation 5.

$$G' = G^* cos \delta \tag{4}$$

(5) $G'' = G^* sin \delta$

G' provides information about the elasticity or the energy stored in the material during deformation, whereas G'' describes the viscous character or the energy dissipated as heat. The combined viscous and elastic behaviours is given by the absolute value of the complex shear modulus G* or by the absolute value of the complex viscosity (η^*). Dynamic conditions were applied to the system to define the phase shift (δ), which is the angle between the values for deformation resulting from sinusoidal stress. A 0° angle indicates that the system is totally elastic while 90° indicates that the system is totally viscous. Intermediate values indicate the viscoelasticity of the system.

The complex viscosity may be obtained from the complex modulus (G*) and the oscillation frequency (w), as shown in Equation 6, which is usually used to evaluate the effect of large deformations and shear rates on the structure of the material.

$$\eta^* = \frac{G^*}{w} \tag{6}$$

Viscoelastic spectra supply information on the elastic (G' x w) and viscous (G' x w) properties. These curves correlate the viscoelastic properties obtained by experimental results with the structures of the colloidal systems.

Other mathematical relationships used to characterize the viscoelasticity were obtained from the dynamic viscosity, shown in Equation 7, and from the phase component of the complex viscosity, shown in Equation 8, (SCHRAMM, 1994; ARRUDA & SANTANA, 1996).

$$\eta' = \frac{G''}{w} \tag{7}$$

$$\eta'' = \frac{G'}{W}$$
(8)

The dynamic viscosity is a measure of the ratio between the energy lost and the frequency in the cyclic deformation. The out of phase component of the complex viscosity is a measure of the ratio between the energy stored and the frequency in the cyclic deformation.

2.3 Response surface

A Central Composite Rotatable Design (CCRD) with two variables, concentration (C) and temperature (T), at 3 levels, -1.414, 0, 1.414, was used to study the response pattern, the rheological parameters (a) and (n), and to determine the

relationship between them (BARROS NETO et al., 1996). Three replicates at the centre point of the design were used to estimate the error. The CCRD combines the vertices of the hypercubes, whose coordinates are given by a 2ⁿ factorial design to provide the estimation of the model curvature.

3. RESULTS AND DISCUSSION

3.1 Viscous results

Experimental data obtained in the steady shear dynamic measurements allowed for the determination of the viscosity and flow curves of the chitosan solutions, as depicted in (Figures 1 and 2), respectively. In Figure 1, the results of the viscosity curves for the chitosan solutions suggest that they behave as pseudoplastic fluids, showing decreasing viscosity values with progressive increases in shear rate. However, in Figure 2, the results for the flow curves also show the time-dependent behaviour of the solutions, which indicates that a classification as thixotropic fluids is more adequate. The thixotropy is affected by the chitosan concentration. At high concentrations, the thixotropy is more evident, with increases in the area between the (+) and (-) curves. This behaviour may be explained by the alignment of the polymeric chains induced by the flow. At higher concentrations, the effect of this alignment becomes more evident, intensely changing the shear stress required for a given deformation (KASAAI et al., 2000). Figure 1 indicates that temperature affects the results more at lower shear rates. These studies allow for the conclusion that the viscosity rise associated with temperature has little importance at higher shear rate values (LAPASIN & PRICL, 1995). This is probably due to the fact that the measurements were made with increasing deformation rate, which may be associated with the alignment of the chains during deformation.



FIGURE 1. Viscosity curves of chitosan solutions as a function of shear rate, at a concentration of 0.025 g.mL⁻¹ and temperatures of 10°C(▼), 30°C(♦) and 50°C(▲).

BRAZILIAN OF FOOD TECHNOLOGY



FIGURE 2. Shear stress of chitosan solutions as a function of increasing (++) and decreasing values (--) for shear rate, at concentrations of 0.025 g.mL⁻¹, 0.03 g.mL⁻¹ and 0.035 g.mL⁻¹ and a temperature of 30°C.

Figures 3 and 4 depict, respectively, the results of the viscosity and flow curves of the gels. The hysteresis is probably a consequence of the modifications that shear causes in the arrangement amongst the chitosan macromolecules, which are cross-linked between themselves. These strong interactions are not found in chitosan solutions (POGODINA et al., 1986). In gels, flow is more time-dependent, suggesting stronger chain structural modifications when compared to chitosan solutions. These structures may reflect irreversible breakage of the tridimensional networks of the gel. In the case of gels, the plateau is a peculiar case amongst the curves. In the case of solutions, an increase in deformation always requires an increase in stress. This behaviour is not observed in the case of gels with high values for shear rate. The cross-links amongst the chains prevent the gels from presenting Newtonian behaviour. These stronger interactions amongst the chains, mainly covalent in the case of gels, are broken with an increase in the shear rate. The area between the curves (+ and -) is also due to these interactions, which are broken during the application of deformation (Figure 3). Therefore the results observed in the flow curves for the gels suggest thixotropic behaviour intensively influenced by intermolecular cross-linking promoted by the glutaraldehyde, as shown in Figure 4 (DESBRIÈRES et al., 1996; DELBEN et al., 1982).

Tables 1 and 2 present the values for the parameters (a) and (n), as well as the correlation coefficient for the Ostwald-de Waele model (R²). The results show that the power law model describes the experimental data for chitosan solutions and gels well. For a given concentration, when the temperature increases, the exponent value (n) increases for the chitosan solutions, behaviour not observed in the gels. This behaviour indicates that chitosan solutions approach Newtonian behaviour with increase in temperature. This behaviour may be explained by increased flexibility of the chains at higher temperatures. The relationship between temperature and flexibility of the polymeric chains may be associated with the torsion angles of the glycosidic linkages and the increase in kinetic energy associated with an increase in temperature. Another effect of temperature is the increase in solvation of the chitosan chains. Decreasing the intensity of the interactions between chitosan chains reflects in lower values for this parameter (a) and it is observed that for a given solution concentration, a rise in temperature leads to lower values. This also reflects an increase in chain mobility with increase in temperature. For concentration, an increase would result in higher values for (a), suggesting an approximation amongst the chains with stronger interactions during flow, causing increasing stress. Such influences can be observed in Table 3, in which the effects are shown. For cross product terms, the value p was considered insignificant and omitted, based on the student t-ratio.



FIGURE 3. Viscosity curves of chitosan gels as a function of increasing (++) and decreasing values (--) for shear rate, at a concentration of 0.025 g.mL⁻¹ and temperature of 30°C.



FIGURE 4. Flow curves of chitosan gels as a function of increasing (++) and decreasing values (--) of shear rate, at a concentration of 0.025 g.mL⁻¹ and temperature of 30°C.

In the case of gels, neither temperature nor concentration affects (a) or (n) as in the case of solutions. This behaviour may be explained by the fact that in gels the chains are covalently bonded and the effect of the deformation is concentrated on the irreversible breakage of parts of these structures (MI *et al.*, 2000). This phenomenon is little influenced by temperature and concentration. Therefore, an increase in the values for (a) and (n) with increase in temperature is not observed in the case of gels. As expected, the values for (a) and (n) depend on the degree of cross-linking.



TABLE 1. Values for the rheological parameters (a) and (n) and the correlation coefficients for the model (R^2) as obtained by linear regression for chitosan solutions.

Concentrations (g.mL ⁻¹)	Temperatures (°C)	а	n	R ²
2.5	10	92.33	0.4376	0.96
2.5	30	45.7	0.5334	0.97
2.5	50	26.44	0.6176	0.96
3	10	145.4	0.4094	0.94
3	30	68.17	0.5219	0.95
3	50	50.58	0.5597	0.96
3.5	10	274.2	0.3309	0.92
3.5	30	120.2	0.4797	0.92
3.5	50	96.45	0.5039	0.95

TABLE 2. Values for the rheological parameters (a) and (n) and the correlation coefficients for the model (R^2) as obtained by linear regression for the gels.

Concentrations (g.mL ⁻¹)	Temperatures (°C)	emperatures a n (°C)		R ²
2.5	10	57.34	0.3746	1
2.5	30	58.85	0.3533	1
2.5	50	50.83	0.3916	1
3	10	48.88	0.3314	1
3	30	57.79	0.3639	1
3	50	52.13	0.3549	0.99
3.5	10	38.72	0.2994	0.97
3.5	30	33.38	0.3517	1
3.5	50	31.17	0.354	0.99

TABLE 3. Effects of the parameters (a) and (n) on the solutions and gels.

Factors —	Effect on	Effect on solution		Effect on gel		
	а	n	а	n		
C.C	37.25	-0,02	-7.4	no		
T.T	-47.41	0.07	no	0.008		
T ² .T ²	29.82	-0.04	-4.27	-0.01		
C ² .C ²	no	no	-7.61	-0.005		

T.T and C.C first order effects; T².T² and C².C² second order effects.

The response surfaces corresponding to Table 3 are presented in Figures 5, 6, 7 and 8, presenting the behaviours of the parameters (a) and (n) for chitosan solutions and gels. In agreement with the experimental design, as mentioned in Section 2, they depend on the values for concentration and temperature. The values for the correlation coefficients (\mathbb{R}^2) for (n) and for (a) and the values for the statistical parameter (F) suggest that the model describe the experimental data well.



FIGURE 5. Response surface showing the effects of concentration and temperature on the rheological parameter (a) of solutions.



FIGURE 6. Response surface showing the effects of concentration and temperature on the rheological parameter (n) of solutions.

3.2 Viscoelastic results

The viscoelastic analyses provided the quantitative evaluation of the viscous and elastic responses for the chitosan solutions and gels. On a logarithmic scale, the viscoelastic spectra are shown in Figures 9 and 10 for the chitosan solutions, while Figures 11 and 12 depict the viscoelastic spectra of the gels. The results obtained for the viscoelastic properties from the spectra shown in Figures 9 and 10, showed the same behaviour found in concentrated solutions of generic polymers, in which the viscous behaviour, represented by the loss modulus G''(w), presented higher values than the elastic behaviour, represented by the storage modulus G'(w) at lower values of frequency. At higher frequencies, the G'(w) becomes higher than G''(w). At



lower frequencies the viscous behaviour is typical of diluted polymeric solutions, in which the chains present in the polymeric structure are weakly linked and the individual chains are free to move almost independently of each other, showing a disordered polysaccharide with a 'random and isolated coils' configuration (MORRIS, 1990; STEFFE, 1996). At high frequencies the complex viscosity decreases sharply with the increase in oscillation frequency. On the other hand, elastic behaviour is typical of polymeric gels, in which individual coils are forced to interpenetrate each other. In this case, the storage of energy by contortion of individual molecules into less energeticallyfavoured chain conformations, becomes increasingly significant, with a consequent increase in G'. This behaviour is observed in Figures 9 and 10. Figure 10 shows crossing between G' and G'' at higher concentrations and lower temperatures, at a lower frequency value. This indicates that torsion of the molecules is an important factor even at the smaller frequency. The results obtained from the viscoelastic spectra shown in Figures 11 and 12 indicate the same behaviour as that of generic polymer gels, the elastic behaviour presenting a higher modulus than for viscous behaviour at any frequency in the range analysed (MORRIS, 1990; STEFFE, 1996). The tridimensional structures are probably strongly linked, approximating to a solid-like behaviour. The introduction of chemical cross-linking amongst the chains led to a stable network and the intrinsic mobility of the molecules decreased dramatically. In the solutions, the networks were connected by chemical interactions such as hydrogen bonding. In the gels, the networks were connected by covalent chemical interaction promoted by the glutaraldehyde. The complex viscosity decreased quickly with the progressive increase in oscillation frequency. The higher storage modulus of the gels as compared to the solutions may be explained by two factors: the covalent bonds present in the chains and the stabilization of the lateral residues due to hydrophobic interactions amongst the polymeric chains (MONTEIRO & AIROLDI, 1999).



FIGURE 7. Response surface showing the effects of concentration and temperature on the rheological parameter (a) of gels.



FIGURE 8. Response surface showing the effects of concentration and temperature on the rheological parameter (n) of gels.



FIGURE 9. Values for G'(++), $G''(\bullet \bullet)$ and $\eta^*(**)$ as a function of the oscillation frequency for chitosan solutions at a concentration of 0.025 g.mL⁻¹ and temperature of 50°C.



FIGURE 10. Values for G'(++), G''(••) and $\eta^*(**)$ as a function of the oscillation frequency for chitosan solutions at a concentration of 0.035 g.mL⁻¹ and temperature of 10°C.



FIGURE 11. Values for G'(++), $G''(\bullet \bullet)$ and $\eta^*(**)$ as a function of the oscillation frequency for gels at a concentration of 0.03 g.mL⁻¹ and temperature of 30°C.



FIGURE 12. Values for G'(++), $G''(\bullet \bullet)$ and $\eta^*(**)$ as a function of the oscillation frequency for gels at a concentration of 0.035 g.mL^{-1} and temperature of 10°C .

The differences between dynamic viscosity (η^*) and shear viscosity (η) indicate deviations from Newtonian behaviour. These deviations supply information about the mobility and flexibility presented by the polymeric chains. These differences are shown in Figures 13 and 14. The values for (η^*) and (η) of the chitosan solutions were very close, behaviour suggesting greater mobility and flexibility since the molecules were further apart and free from each other (ATKINS, 1985). In this case the complex viscosity can be expressed by (η') . On the other hand, for chitosan gels the values for (η^*) and (η) values were considerably different from each other because of the formation of the tridimensional networks. The increasing deviation from Newtonian behaviour may be justified due to the new interactions introduced into the system (JIANG et al., 1999). In this case, the complex viscosity can be expressed by (η´´).



FIGURE 13. Shear viscosity as a function of shear rate and complex viscosity as a function of oscillation frequency for chitosan solutions at a concentration of 0.025 g.mL⁻¹ and temperature of 30°C.



FIGURE 14. Shear viscosity as a function of shear rate and complex viscosity as a function of oscillation frequency for gels at a concentration of 0.025 g.mL⁻¹ and temperature of 30°C.

4. CONCLUSIONS

The viscous behaviour of the chitosan solutions and gels may be described satisfactorily by the Ostwald-deWaele model, time-independent behaviour, with influence of the variables temperature and concentration on the parameters (a) and (n). Therefore, the thixotropic behaviour was also observed, mainly in the case of the gels, which indicates that a classification as thixotropic fluids is more adequate. The results suggest that the thixotropic behaviour is not only caused by orientation but also by molecule-interaction. Despite being a polysaccharide, the chitosan solutions presented the same behaviour verified for concentrated solutions of generic polymers, while the gels presented the same behaviour observed for the gels of generic polymers. It was observed that all the viscoelastic properties measured, including G', G" and η^* , were significantly influenced by the effect of the chemical composition on the solution and on the gelled state. For chitosan solutions, η^* and η were very close, suggesting high mobility and flexibility. For chitosan gels,



 η^* and η were completely different because of the formation of tridimensional networks. The experimental results reported may contribute as a support for the design of equipment used in the handling of the biopolymer chitosan in several applications

ACKNOWLEDGEMENTS

The authors acknowledge Professor Luiz Benedicto Pompeo Neto for valuable discussions and CAPES for financial support.

REFERENCES

- ARRUDA, E. J.; SANTANA, C. C. Viscoelasticidade de géis de colágenotipo-1. Tese de Mestrado, FEO-Unicamp, 1996.
- ATKINS, E. D. T. **Polysaccharides-Topics in Structure and Morphology**, Weinheim, USA, 1985, 223 p.
- BARROS NETO, B. *et al.* **Planning and Optimization of Experiments**, 1ed. Unicamp publisher, 1996, 299 p.
- BEPPU, M. M.; SANTANA, C. C. Estudo da calcificação in vitro de quitosana. Dr. Thesis, School of Chemical Engineering, UNICAMP, 1999, 150p.
- CHIRACHANCHAI, S. *et al.* Carbaryl insecticide conjugation onto chitosan via iodochitosan and chitosan carbanyl imidazolide. **Carbohydrate Polymers**, v. 46, n. 1, p. 19-27, 2001.
- DELBEN, F. et al. Rheology of aqueous N-(carboxymethyl) chitosan systems. Carbohydrate Research, v. 10, n. 1, p. 199-214, 1982.
- DESBRIÈRES, J. *et al.* Hydrophobic derivatives of chitosan: characterization and rheological behaviour. **Biological Macromolecules**, v. 19, n. 1, p. 21-28, 1996.
- DRAGET, K. I. Associating phenomena in highly acetylated chitosan gels. **Polymer Gels and Network**, v. 4, n. 1, p. 143-151, 1996.
- HUANG, R. Y. M. *et al.* N-acetylated chitosan membranes for the pervaporation separation of alcohol/toluene mixtures. **Journal of Membrane Science**, v. 176, n. 2, p. 223-231, 2000.

- JIANG, H. *et al.* Rheology of highly swollen chitosan/polyacrylate hydrogels. **Polymer**, v. 40, n. 16, p. 4593-4602, 1999.
- KASAAI, M. R. *et al.* Master curve for concentration dependence of semi-dilute solution viscosity of chitosan homologues: the Martin equation. Food Research International, v. 33, n. 1, p. 63-67, 2000.
- LAPASIN, R.; PRICL, S. Rheology of Industrial Polysaccharides: Theory and Applications, 1 ed. Blackie London, 1995, 620p.
- LEE, S. T. *et al.* Equilibrium and kinetic studies of copper (II) ion uptake by chitosan-tripolyphosphate chelating resin. **Polymer**, v. 42, n. 5, p. 1879-1892, 2001.
- MI, F. L. *et al.* The study of gelation kinetics and chain-relaxation properties of glutaraldehyde-cross-linked chitosan gel and their effects on microspheres preparation and drug release. **Carbohydrate Polymers**, v. 41, n. 4, p. 389-396, 2000.
- MONTEIRO, O. A. C.; AIROLDI, C. Some studies of crosslinking chitosanglutaraldehyde interaction in a homogeneous system. **Biological Macromolecules**, v. 10, n. 1, p. 120-129, 1999.
- MORRIS, E. R. The Structure, Dynamics and Equilibrium Properties of Colloidal System, 1 ed. Oxford, London, 1990, 470 p.
- MUSLIM, T. *et al.* Synthesis and bioactivities of poly(ethylene glycol)chitosan hybrids. **Carbohydrate Polymers**, v. 46, n.4, p. 323-330, 2001.
- MUZZARELLI, R. A. A. Chitin Handbook, 1 ed. Italy Plenum, 1977, 500p.
- POGODINA, N. V. *et al.* Conformational characteristics of chitosan molecules as demonstrated by diffusion-sedimentation analysis and viscometry. **Polymer Science**, v. 28, n. 2, p. 251-259, 1986.
- ROBERTS, G. A. F. Chitin Chemistry, The Macmillian Press Ltd, London, 1992, 300p.
- SCHRAMM, G. A Practical Approach to Rheology and Rheometry. Germany: HAAKE, 284 p., 1994.
- SMIDSROD, O. *et al.* Preparation and characterization of chitosans with oligosaccharide branches. **Carbohydrate Research**, v. 337, n. 24, p. 2455-2462, 2002.
- STEFFE, J. F. **Rheological Methods in Food Process Engineering**, 2 ed. Michigan Freeman Press, 1996, 412 p.
- TANGPASUTHADOL, V.; PONGCHAISIRIKUL, V. Surface modification of chitosan films: effects of hydrophobicity on protein adsorption. Carbohydrate Research, v. 338, n. 9, p. 937-942, 2003.