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The effectiveness of the protected amino group on crosslinked chitosans for copper removal and the thermodynamics of interaction at the solid/liquid interface

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ABSTRACT

Acidic glutaraldehyde (Gh) crosslinked chitosan (ChGhH) when deprotonated the biopolymer (ChGh) presents high content of free amino groups. These modified biopolymers are comparable to epichlorohydrin (Ep) crosslinked (ChEp). C/N molar ratio of 6.1 for chitosan increases to 7.3, 7.5 and 7.1 for ChGhH, ChGh and ChEp. The effectiveness of the carbon-6 hydroxyl group in interconnecting chitosan units was supported by IR and ^{13}CNMR, where Ep promotes increase in crystallinity. Copper uptake gave the order Ch > ChGh > ChGhH > ChEp, as: 1.35 ± 0.06, 1.30 ± 0.05, 1.05 ± 0.07 and 0.96 ± 0.22 mmol g^{-1}, reflecting the availability of nitrogen basic centers in adsorbing. The favorable thermodynamic data of adsorption through calorimetric titration gave exothermic enthalpic values: −28.98 ± 0.05, −6.68 ± 0.04, −6.13 ± 0.07 and −0.65 ± 0.23 kJ mol^{-1} for Ch, ChGh, ChGhH and ChEp. Free Gibbs energy reflected spontaneity of interactions and, with the exception of chitosan, the entropic values are positive.

1. Introduction

The natural biopolymer chitin, extractable from crustacean shells and insect cuticles, yields chitosan when N-deacetylated in a thermal alkaline process (Guibal, 2005; Vasiliu, Popa, & Rinaudo, 2005). The precursor chitin and chitosan have identical linear copolymeric structures constituted by two monomers: 2-acetamido-2-deoxy-D-glucopiranose units and the deacetylated 2-amino-2-deoxy-D-glucopiranose form. Both biopolymers are normally distinguishable through the deacetylation degree (DD) property, as a useful quantitative datum related to the percentage of deacetylated units (Lima & Airoldi, 2004; Vold & Christensen, 2005). Considering this property, there is no formal nomenclature; however, it is expected that chitosan denotes a DD value higher than 50% (Brugnerotto et al., 2001; Chenite, Buschmann, Wang, Chaput, & Kandani, 2001).

Nowadays, chitosan and its derivatives have been extensively investigated in recent years due to them intrinsic associated properties, such as non-toxicity, high biocompatibility and biodegradability, in addition to attractive physical and mechanical domains. The low cost and relatively easy preparative methods give to these biopolymers many potential applications in several fields, not only academic, but also for practical uses in medicine, agriculture, food, pharmaceutical technology, cosmetics and so on (Jayakumar, Reis, & Mano, 2006; Kurita, 2006).

From the reactivity viewpoint, chitosan commonly forms Schiff bases or imines, when reacting with aldehydes or ketones, to form at least one N=C group, due to the condensation reaction of the primary amine with the carbonyl group (Sashiwa & Aiba, 2004). Under certain favorable conditions, chitosan forms a three-dimensional network (Berger et al., 2004), as the linear single polymeric chains are progressively interconnected, as a result of crosslinking process. The success of such interactive procedures depends on the choice of the appropriate crosslinking agent (Lin, Yu, & Yang, 2005; Shu & Zhu, 2002), since amino or hydroxyl groups will necessarily be involved in chemical modifications. The new biopolymer reflects in changed mechanical properties, selectivities, adsorption capacities, or even to prevent dissolution in acidic medium, a desirable property for biological system applications (Shu & Zhu, 2002). Among a series of uses of crosslinked chitosans, a great number of studies have been devoted to metal removal, mainly those concerning to divalent cations (Airoldi & Monteiro, 2003). Crosslinkers may include bi or polyfunctional agents, being the most useful agents sodium tripolyphosphate, ethyleneglycol diglycidyl ether, glutaraldehyde and epichlorohydrin; these last two have been intensively explored recently (Coelho, Laus, Mangrich, Fávere, & Laranjeira, 2007).

The distinctive features associated with these two bifunctional crosslinking agents, epichlorohydrin (chloromethyloxirane) and glutaraldehyde, are related to the reactivity of the functional groups attached to the polymeric structure. Thus, the first is widely employed as an intermediate for synthesis in chemical industries. In the course of the reaction, the epoxide ring is opened to establish a bonded OH group, to preserve the basic character of the

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chitosan biopolymeric structure, with great advantage in leaving free amino groups on the main backbone skeleton (Coelho et al., 2007). On the other hand, glutaraldehyde has also been extensively applied as a crosslinking agent, due to its low cost, with high water solubility and enormous ability to react with available amino functions, to establish a bridge between linear polymeric chains. Such interaction normally involves primary amino groups to yield a Schiff base in an intermolecular fashion, by generating the three-dimensional structure through covalent bond formation (Vieira & Beppu, 2006). However, when amino groups are chemically protected, the aldehyde function also has ability to react with hydroxyl groups on the linear polymeric structure. Therefore, when a subsequent reaction step is carried out and the protecting group is removed, the amino function is restored as free unit to react in the next step.

The present investigation deals with synthesized crosslinked chitosan using epichlorohydrin as well as glutaraldehyde molecules. This latter agent was explored in two distinct conditions: (i) to promote different crosslinking modes, on hydroxyl or (ii) on amino groups; this process depends on the protecting effect on the amino groups under acidic conditions. The obtained biomaterials were applied for copper removal from aqueous solution, and the interactions between the metal and the basic centers at the solid/liquid interface were followed by calorimetry, to determine the thermodynamics involved in the interactive processes.

2. Materials and methods

2.1. Chemicals

Chitosan (Ch) extracted with a degree of acetylation of 82% was used. Glutaraldehyde (Gh), acetone, hydrochloric acid, epichlorohydrin (Ep), sodium hydroxide and copper nitrate were all analytical reagents. Deionized water was used in all experiments.

2.2. Equipment and measurements

The percentages of carbon and nitrogen in the chitosan and the crosslinked biopolymers were determined through elemental analysis on a Perkin Elmer, model 2400, elemental analyzer. Infrared spectra of the samples as KBr pellets were obtained by accumulating 32 scans on a Bomem Spectrophotometer, MB-series, in the 4000–400 cm^{-1} range, with 4 cm^{-1} of resolution, by applying Fourier transformation. Solid state ^{13}C NMR spectra of the samples were obtained on a Bruker AC 300/P spectrometer. The measurements were obtained at 75.47 MHz, with a magic angle spinning of 4 kHz. The CP/MAS technique was used, with pulse repetition of 5 s and contact time of 1 ms. X-ray diffraction patterns were obtained on a Shimadzu XD-3A diffractometer (35 kV, 25 mA), in the $2\theta = 1.5$ to 50° range using nickel-filtered CuKα radiation, with a wavelength of 0.154 nm. Thermogravimetric curves were obtained using a Shimadzu TGA 50 apparatus, under an argon atmosphere at a flow rate of 1.67 $cm^3\ s^{-1}$ and heating rate of 0.167 $K\ s^{-1}$. The amount of cation adsorbed was determined by the difference between its initial concentration in the aqueous solution and that found in the supernatant, by using a Perkin Elmer 3000 DV ICP-OES apparatus. For each experimental point, the reproducibility was checked by at least one duplicate run.

2.3. Crosslinking methodology

A sample of chitosan (10.0 mmol), whose mass was calculated taking into account the amount of free amino groups, determined from the degree of deacetylation, was added to a conical flask containing hydrochloric acid (12.0 mmol) followed by glutaraldehyde (12.0 mmol) in acetone (100 cm^3), under magnetic stirring. The aim of adding hydrochloric acid was to protect the NH_2 groups in the crosslinking reaction with the available aldehyde (Kim, Kim, Jegal, & Lee, 2003). After 16 h, the solid was separated by filtering and extensively washed with deionized water to neutrality of the filtered factions and the isolated solid, hereafter named ChGhH, was dried at 333 K, in an oven. In a second step, the proton was removed from ChGhH by immersing it in 0.24 mol dm^{-3} sodium hydroxide in water/acetone (1:1) solution for 24 h, under stirring. The product of this reaction, named ChGh, was dried as previously. From this operation a new crosslinked chitosan resulted that again should be able to react through the available amino groups. Then, both ChGhH and ChGh biomaterials were applied for copper uptake from aqueous solution in order to compare their adsorptive capacities.

Another crosslinking reaction was carried out with the epichlorohydrin (Ep) agent due to its high ability for this operation (Ngah, Endud, & Mayanar, 2002). Briefly, chitosan was added to an epichlorohydrin solution in 1.0×10^{-3} mol dm^{-3} sodium hydroxide solution, to establish chitosan/epichlorohydrin molar ratio 1:1, and the reaction was maintained under stirring during 3 h at 318 K. The isolated solid (ChEp) was separated, washed with deionized water until neutral pH and dried at 333 K. This biopolymer was also used as adsorbent for copper recovery from aqueous solution.

2.4. Adsorption

All sorption experiments were performed in duplicate, using a batch process in which nearly 20 mg of chitosan or each chemically modified chitosan were suspended in a series of polyethylene flasks containing 25.0 cm^3 of copper (II) solution, having concentrations ranging from 7.0×10^{-4} to 7.0×10^{-3} mol dm^{-3}. The suspensions were shaken for 6 h in an orbital bath at 298 ± 1 K. This time was previously established by using the same procedure, to obtain an isotherm with a well-defined plateau, indicating that all basic centers in each biopolymer were saturated by cations. The supernatant solutions were separated from the solids by decantation and aliquots were taken to determine the amounts of copper remaining by the ICP-OES technique. The amount of the cation adsorbed during the experimental assays (mmol dm^{-3}) was calculated by Eq. (1), where N_f is the number of moles adsorbed on pristine or crosslinked chitosans, n_i and n_s are the number of moles in the initial solution and in the supernatant after equilibrium, and m is the mass of the adsorbent used in each adsorption process (Lima & Airoldi, 2003).

$$N_f = (n_i - n_s)/m \tag{1}$$

The experimental data related to the number of moles in the supernatant in each point of the titration, C_s, and the N_f obtained were fitted to the modified Langmuir equation (Eq. (2)) in order to determine the maximum adsorption capacity, N_s, with b being a constant related to the chemical equilibrium at the solid/liquid interface.

$$\frac{C_s}{N_f} = \frac{1}{N_{sb}} + \frac{C_s}{N_s} \tag{2}$$

2.5. Calorimetric titration

The interactions between the cation and the basic centers attached on the chitosans were measured through calorimetric titrations on a LKB 2277 instrument (Macedo & Airoldi, 2006), where three independent titrations must carried out to complete the thermodynamic cycle: (i) the thermal effect due to chitosan

or chemically modified chitosans interacting with copper (II) (Q_{tit}), (ii) hydration of the solid biopolymers (Q_s) and (iii) dilution of copper solution (Q_{dil}). For the Q_{tit} experimental titration, the metallic solution is added to a suspension of 20 mg of each biopolymer sample in 2.0 cm^3 of water, under stirring at 298.15 ± 0.20 K. Increments of 10.0×10^{-3} cm^3 of copper (II) solution were added to each biopolymer to obtain the thermal effect of interaction (Q_{tit}), however, the thermal effect of hydration of the suspended solid samples in water was null (Macedo & Airoldi, 2006). Thus, the resulting thermal effect is given by the following equation:

$$\sum Q_{res} = \sum Q_{tit} - \sum Q_{dil} \tag{3}$$

After adjusting the adsorption data to a modified Langmuir equation it is possible to determine the enthalpy associated with the cation/biopolymer interaction and the enthalpy of the formation of a monolayer per unit of mass of adsorbent, $\Delta_{mono}H$, can be determined:

$$\frac{\sum X}{\sum \Delta H} = \frac{1}{(K-1)\Delta_{mono}H} + \frac{X}{\Delta_{mono}H} \tag{4}$$

where $\sum X$ is the sum of the mole fractions of the cation solution after adsorption, and X is obtained for each point of titrant addition; ΔH (J/mol), the enthalpy of adsorption per gram of adsorbent, is obtained by dividing the thermal effect resulting from adsorption by the number of moles of adsorbate and K is the proportionality constant, which also includes the equilibrium constant. From angular and linear values from the $\sum X/\sum \Delta H$ versus $\sum X$ plot it is possible to calculate the Δ_{mono}H value. Then, the enthalpy of adsorption, ΔH can be calculated through Eq. (5):

$$\Delta H = \frac{\Delta_{mono}H}{n^s} \tag{5}$$

From K values, the Gibbs free energies (ΔG) were calculated through:

$$\Delta G = -RT \ln K \tag{6}$$

and the entropy (ΔS) can be calculated:

$$\Delta G = \Delta H - T\Delta S \tag{7}$$

3. Results and discussion

3.1. Elemental analysis

The precursor chitosan and all chemically crosslinked biopolymers, whose schematic synthetic procedures involving glutaraldehyde and epichlorohydrin are shown in Fig. 1, were elementally analyzed. Based on the percentages of carbon and nitrogen, the carbon/nitrogen relationships were calculated and the results are listed in Table 1.

The data show an increase in carbon/nitrogen molar ratio from chitosan to the crosslinked biopolymers, in complete agreement with the inclusion of the crosslinker molecules to form new biopolymers. Thus, as each molecule progressively crosslinked two independent biopolymeric chains, the amount of carbon atoms increases reflecting in higher values, due to the absence of nitrogen atom in these agents, varying from 7.1 to 7.3, which are higher than that found for chitosan, a 6.1 value.

3.2. Infrared spectroscopy

The infrared spectrum of chitosan is exhibited in Fig. 2, which also contains the spectra of the crosslinked chitosans. The characteristic chitosan bands are C—H stretching around 2900 cm^{-1} and a broad and intense band near 3400 cm^{-1} corresponding to O—H and

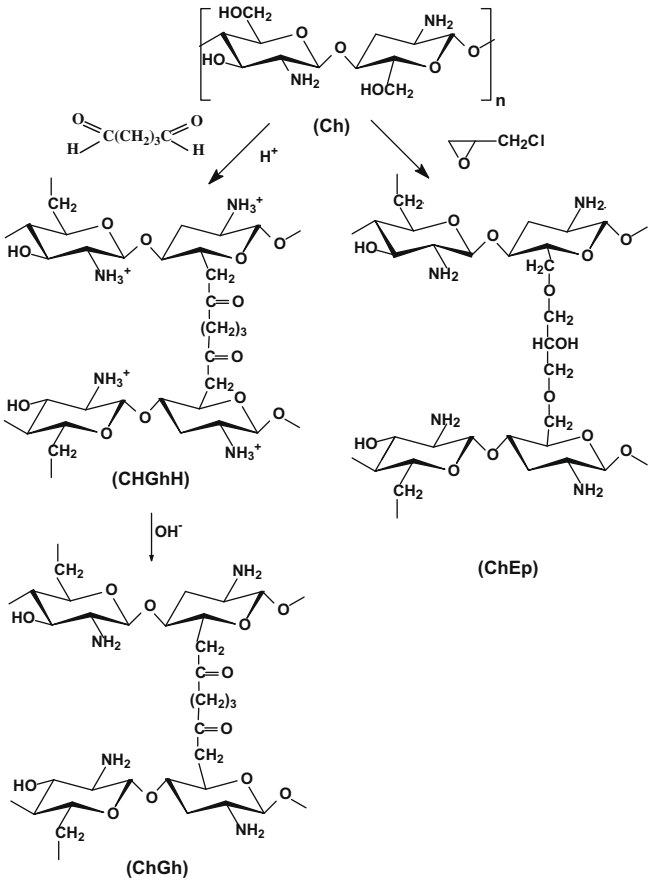

Fig. 1. Chitosan crosslinked with glutaraldehyde under acidic conditions (ChGhH), followed by deprotonation (ChGh) and chitosan crosslinked with epichloroydrin (ChEp).

Table 1
Percentages of nitrogen (N) and carbon (C), the respective number of moles and C/N molar ratio for chitosan and the crosslinked biopolymers.

Sample	C/%	N/%	C/mmol g^{-1}	N/mmol g^{-1}	C/N
Ch	40.43	7.69	33.8	5.5	6.1
ChGh	41.12	6.48	34.3	4.6	7.5
ChGhH	39.47	6.29	32.9	4.5	7.3
ChEp	39.79	6.61	33.2	4.7	7.1

N—H stretchings. The bands corresponding to acetamide group, remaining from chitin, due its incomplete deacetylation (Laus et al., 2006), appear at around 1655 and 1380 cm^{-1}, and are attributed to C=O and C—H deformations. The band at 1320 cm^{-1} is related to aliphatic C—H bending vibrations and those between 2920 and 2850 cm^{-1} correspond to the stretching vibrations of the same groups. The bands in the 1200–800 cm^{-1} region are associated with the pyranosidic ring, reflecting C—O—C and β-glycosidic linkage as well as the C—O related to primary and secondary alcohols. The spectra of all chemically modified biomaterials showed slight changes when compared to the original chitosan, which may be explained by considering the structure of the crosslinking agents, as observed for both Gh and Ep, which include CH_2 groups. From Gh crosslinking, a carbonyl function was introduced and an alcohol function was added in the Ep reaction. It is important, however, to note that the functional groups introduced by incorporating both crosslinker moieties are also present in the original skeleton, so, it is reasonable that no significant changes are expected for the corresponding spectra after the reaction.

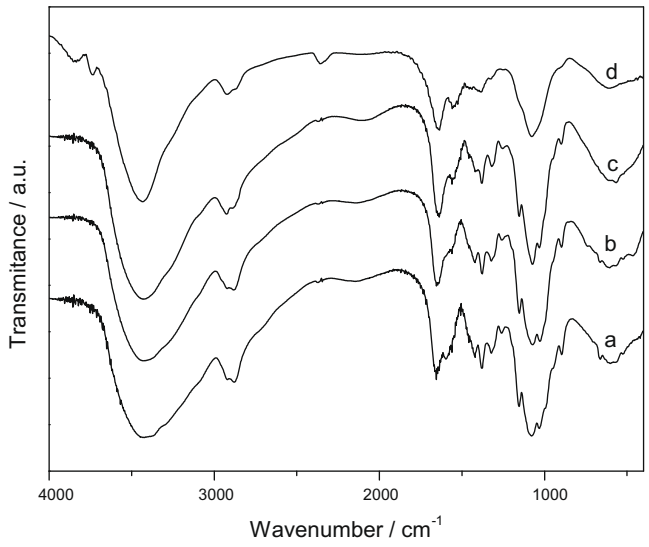

Fig. 2. Infrared spectra of chitosan (a), ChEp (b), ChGhH (c) and ChGh (d) biopolymers.

3.3. X-ray diffraction

The X-ray diffraction patterns demonstrated poor crystallinity for chitosan, a fact that is denoted by the presence of two broad peaks around 9° and 20°, as previously reported (Monteiro & Airoldi, 1999). When chitosan was chemically modified with glutaraldehyde, both protonated and unprotonated forms presented a decrease in crystallinity, similar results were detected with chitosan crosslinked with glutaraldehyde (Monteiro & Airoldi, 1999). On the contrary, when binding to the epichlorohydrin molecule the resulting biopolymer has a slightly enhanced in crystallinity, as suggested by the well-formed peaks, illustrated by ChEp in Fig. 3. For this set of compounds, the crystallinity decreased in the following order: ChEp > Ch > ChGhH > ChGh. The crystallinity associated with chitosan is closely related to the intra and intermolecular hydrogen bond system involved in all polymeric chains to maintain their stability. As an appropriate reagent has the ability to disrupt this set of bonds during the course of the reaction, in general, the new chemically modified chitosan decreases in crystallinity in comparison with the original chitosan. Thus, the crystallinity of chitosan is a key parameter in the accessibility of internal sites for water as well as metal ions, since such kind of reactions will not occur without breaking these well-formed linkages (Qi & Xu, 2004).

3.4. NMR spectroscopy

^{13}C NMR spectra of chitosan and all derivatives are shown in Fig. 4. The characteristic chemical shifts for pristine chitosan are found at 105, 55, 85 and 60 ppm, related to the C1, C2, C4 and C6 carbons, respectively, as well as the peak at 75 ppm attributed to C3,5 carbons. The signals at 22 and 175 ppm are related to methyl and carbonyl groups from the original chitin, as expected due to incomplete deacetylation, as indicated by the labeled carbon atoms in the inserted skeleton structure of then biopolymer shown in Fig. 4a. ChEp, Fig. 4d, presented C1, C3,5 and C6 signals enlarged, due to the overlapping with the neighboring C4 peak and making the C2,6 and C3,5 signals slightly separated, when compared to the unmodified chitosan. The derivative ChGdH shown in Fig. 4c exhibited similar behavior, but in this case, the C2 peak overlaps the C6 signal, as was observed previously after the crosslinking reaction in chitosan hybrids (Monteiro & Airoldi, 1999). The peaks in the 30–50 ppm region suggest different chemical environments for CH_2 groups. Such different environments are due to the contribution of the glutaraldehyde and epichlorohydrin molecules to the new polymeric structures. After reaction with sodium hydroxide, the bonded proton was removed from the ChGhH derivative, to give a free amino group, which was regenerated from the polymeric structure. Then, the C4 signal reappeared and the most significant modification was found in the related C6 and C2 peaks, which were completely separated on the ChGh stpectrum, indicating that the chemical environment was modified due the introduction of new fragments in the chitosan polymeric chain, as shown in Fig. 4b. As observed, the chemical change affected the linear chain distribution, reflecting in some carbon environmental structures.

3.5. Thermogravimetry

The thermal degradation profile for curves is similar for original chitosan and their derivatives. The thermogravimetric curves are

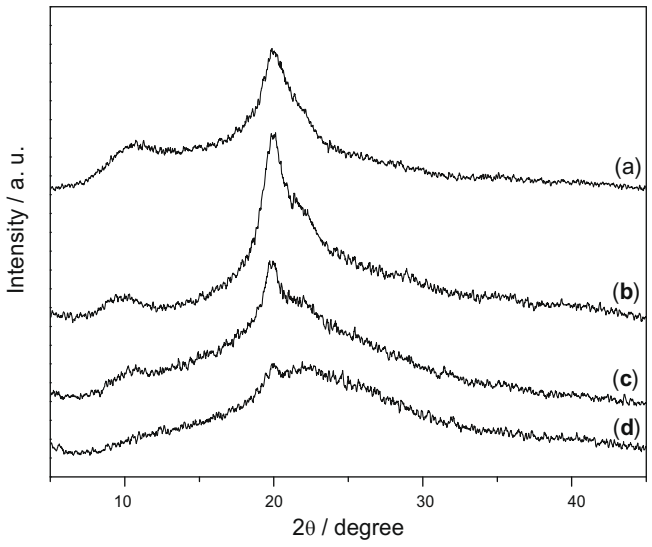

Fig. 3. X-ray diffraction patterns of chitosan (a), ChEp (b), ChGhH (c) and ChGh (d) biopolymers.

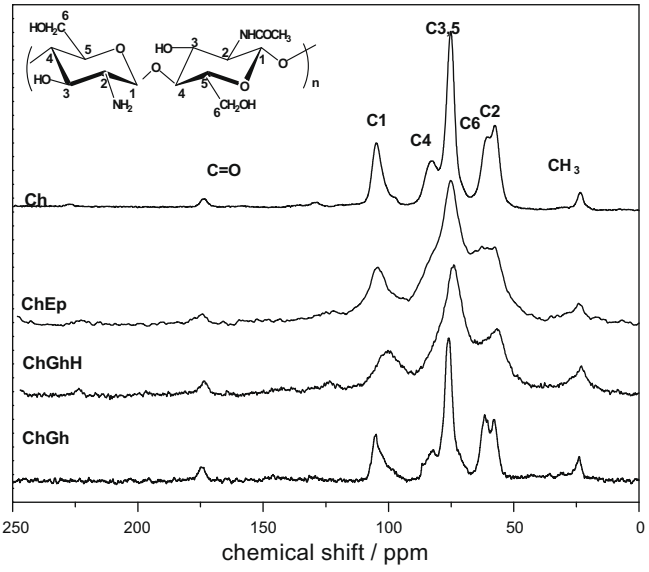

Fig. 4. ^{13}C NMR spectra in the solid state for chitosan (a), ChEp (b), ChGhH (c) and ChGh (d) biopolymers.

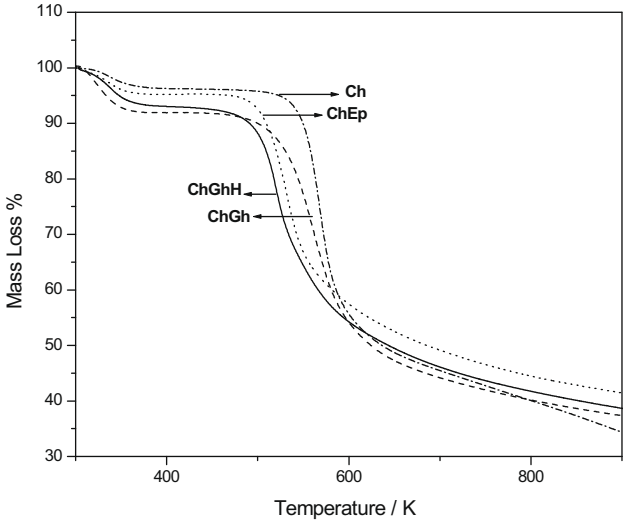

Fig. 5. Thermogravimetric curves of chitosan (Ch), ChEp, ChGhH and ChGh.

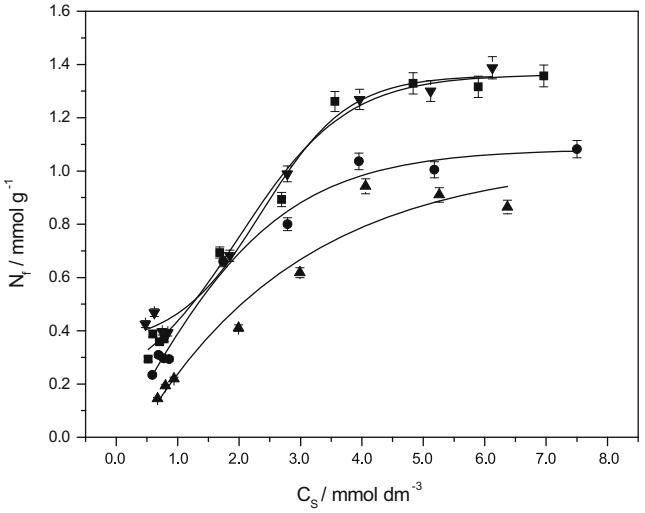

Fig. 6. Adsorption isotherms of copper on chitosan (■), ChEp (▲), ChGhH (●) and ChGh (▼) at 298 ± 1 K.

shown in Fig. 5 and the decomposition suggested that the degradations occurred in two stages. The first stage is attributed to loss of adsorbed water that is observed near 330 K for chitosan and all derivatives. The second mass loss for original chitosan occurred at 568 K, whereas for the derivatives, the corresponding peaks were at 533, 520 and 562 K for ChEp, ChGhH and ChGh, respectively. Similar profile decompositions were observed before for chitosan and the biopolymer modified with glycolic and lactic acids (Qu, Wirsen, & Albertsson, 2000). These values represent the maximum mass loss temperature of the synthesized polymeric biomaterials and were obtained from the first derivative of the thermogravimetric plots. These results reflect that chitosan is more stable than the derivatives at temperatures lower than 600 K. However, all crosslinked biomaterials presented lower mass losses than pristine chitosan in this temperature range, suggesting an increase in thermal properties of the chemically modified chitosans, when compared to the original biopolymer.

3.6. Adsorption isotherms

The ability of chitosan to extract cations has been previously explored and copper ion has been widely employed in adsorption assays, since it is normally adsorbed by the natural biopolymer from aqueous solution in neutral conditions (Monteiro & Airoldi, 1999). The set of isotherms involving chitosan and all chemically modified compounds with copper are shown in Fig. 6. As observed, chitosan presented adsorption capacity very close to the ChGh derivative, to give 1.35 ± 0.06 and 1.30 ± 0.05 mmol g^{-1}, respectively, while for other chemically modified biopolymers ChGhH and ChEp the values of 1.05 ± 0.07 and 0.96 ± 0.22 mmol g^{-1} were obtained. From the coordination point of view it was well established that chitosan has the ability to bind copper cation through electron pairs available on the nitrogen atom in the free amino groups (Monteiro & Airoldi, 1999), which varied as a function of the effectiveness of the crosslinker agents and, in the present case, with amine group protonation.

The crosslinking agent epichlorohydrin interconnects two neighboring linear polymeric chains by bonding to hydroxyl groups on carbon 6. After establishing this molecular arrangement the amino groups remain free to interact with cations, from which the low adsorption capacity for ChEp is unexpected, as shown in Fig. 1. However, another feature to be considered is related to the length of the crosslinking chains. The shorter epichlorohydrin molecule could be responsible for a rigid polymeric chain in the final reaction product, in comparison with the longer glutaraldehyde molecule. This fact can explain the reduced affinity of cation to interact with the basic centers on the well-formed polymeric structure, with a consequent decrease in adsorption ability, as given by 0.96 ± 0.22 mmol g^{-1} value.

The behavior of both glutaraldehyde derivatives were affected by the synthetic route and depend on the free amino groups, with the ChGhH polymer showing a lower performance, compared to chitosan, due the fact that the basic nitrogen center is blocked by protons, to give an adsorption of 1.05 ± 0.07 mmol g^{-1}. On the other hand, it was expected that the adsorption capacity would be enlarged after the deprotecting step, to give 1.30 ± 0.05 mmol g^{-1}. This new derivative resembles the natural biopolymer, since the amino groups were restored by the deprotonating process and, consequently, the chemically modified chitosan has an identical free amino group content, to be able to chelate copper. These results suggest that the use of glutaraldehyde as crosslinking agent, in an acidic reaction, following by deprotonation, allows achieving advantages of crosslinked material, without loosing the adsorption capacity of the polymeric chitosan. So, it was found that those chitosans presented the following order of adsorption: Ch > ChGh > ChGhH > ChEp. Furthermore, the above results with respect to the chemically modified chitosans are consistent with the X-ray diffraction patterns, since the decrease in crystallinity is related to hydrogen bond disruption and favor the access of the cation to reach the available basic centers. Based on the present set of results, a novel feature to be expressed is that the adsorption capacity varies inversely with the crystallinity of the crosslinked chitosans.

3.7. Calorimetric titration

The thermal effects obtained from copper nitrate interaction with precursor chitosan and their derivatives were determined in separate calorimetric experiments. The quantitative net effects for each biopolymer/copper interaction were obtained after subtracting the cation dilution effect, as given by Eq. (3). These thermal effects from the complete thermodynamic cycle for this series of interactions involves a suspension (sp) of biopolymers (Biop) in aqueous (aq) solution with copper ion (Cu^{2+}) are represented as follows:

Table 2
Summary of the thermodynamic values for copper/biopolymer interactions at the solid/liquid interface at 298.15 ± 0.20 K.

Sample	$-\Delta_{mono}H$/J g^{-1}	$-\Delta H$/kJ mol^{-1}	ln$K$	$-\Delta G$/kJ mol^{-1}	ΔS/J mol^{-1} K^{-1}
Ch	11.10 ± 0.07	28.98 ± 0.05	8.5	21.1 ± 0.1	−26 ± 1
ChGh	11.62 ± 0.02	6.68 ± 0.04	11.24	27.8 ± 0.1	71 ± 1
ChGhH	9.44 ± 0.05	6.13 ± 0.07	10.28	25.4 ± 0.1	64 ± 1
ChEp	3.03 ± 0.13	0.65 ± 0.23	11.71	29.0 ± 0.1	95 ± 1

$$\mathrm{Biop}_{(sp)} + \mathrm{Cu}^{2+}_{(aq)} = \mathrm{Biop}\cdot\mathrm{Cu}^{2+}_{(sp)};\quad Q_t \tag{8}$$

$$\mathrm{Biop}_{(sp)} + n\mathrm{H_2O} = m\cdot n\mathrm{H_2O}_{(sp)};\quad Q_h \tag{9}$$

$$\mathrm{Cu}^{2+}_{(aq)} + n\mathrm{H_2O} = \mathrm{Cu}^{2+}\cdot n\mathrm{H_2O}(aq);\quad Q_d \tag{10}$$

$$\mathrm{Biop}\cdot n\mathrm{H_2O}_{(sp)} + \mathrm{Cu}^{2+}\cdot n\mathrm{H_2O} = \mathrm{Biop}\cdot\mathrm{Cu}^{2+}_{(sp)} + 2n\mathrm{H_2O};\quad Q_r \tag{11}$$

The great advantage of the calorimetric titration for this heterogeneous system is the simultaneous determination of the equilibrium and enthalpy data, after adjusting the net thermal effects to a mathematical model. Through these values, the Gibbs free energy and the entropy for this system can be also calculated. For the present case, the net thermal effect obtained from the calorimetric titration is given by the expression $\Sigma Q_r = \Sigma Q_t - \Sigma Q_d$, based on Eq. (11). By applying Eq. (4) and the linearized data for all biopolymers, the enthalpy involved in the formation of a monolayer, $\Delta_{mono}H$, can be obtained for all processes, which enable the calculation of the molar enthalpy, as given in Eq. (5). From the K value the Gibbs free energy was calculated from Eq. (6) for cation–basic center interactions on all chitosans and the thermodynamic entropic value was calculated from enthalpic and Gibbs free energy values through Eq. (7) and the complete set of values are listed in Table 2.

The enthalpic results −28.98 ± 0.05, −6.68 ± 0.04, −6.13 ± 0.07 and −0.65 ± 0.23 kJ mol^{-1} are exothermic for all biopolymers and based on the magnitude of the following order is presented: Ch > ChGh > ChGhH > ChEp. This behavior can be directly associated with the availability of the free amino groups to interact with cations at the solid/liquid interface. These enthalpic values clearly reflect the success of the crosslinking process by giving the highest value for Ch, while the crosslinker agents reduced the availability of the amino groups to react with cation, and consequently, decreased the enthalpy of the system. The enthalpy of copper/amino group interaction reflects the influence of the proton on crosslinked biopolymers, as expressed by the values for free and protonated biopolymers, with −6.68 ± 0.04 and −6.13 ± 0.07 kJ mol^{-1}, respectively. Although epichlorohydrin crosslinking biopolymer presented free amino groups for copper coordination, its low enthalpic value support the proposal that the shorter chain cause difficulty for the cation to interact with the potential available basic centers, to give the lowest value in this series.

The negative Gibbs free energy values for chitosan and their derivative forms indicate that a spontaneous process of complexation for copper by chitosan takes place, as previously observed (Lima & Airoldi, 2004). Pure chitosan gave a negative entropic value, which is also consistent with previously reported results (Monteiro & Airoldi, 1999, 2005) and this fact is interpreted that copper/chitosan interactions cause an ordering of the water in this system upon complexation. However, for chemically modified biopolymers a contrary behavior was observed, in which disorder is caused by the displacement of water molecules to the medium. This behavior could be associated with the increase in hydrophobicity of the final biopolymer formed, as two independent chains are crosslinked through the insertion of these agents between two subsequent polymeric chains.

4. Conclusion

The syntheses of crosslinking biopolymers based on chitosan and the crosslinker epichlorohydrin and glutaraldehyde agents were efficiently performed, according to infrared and carbon nuclear resonance spectroscopies. X-ray diffraction patterns reveal that modifications on chitosan chains yield crosslinked biopolymers with poor crystallinity. The chemically modified compounds adsorbed copper giving the values 1.35, 1.30, 1.05 and 0.96 mmol g^{-1} for Ch, ChGh, ChGhH and ChEp, respectively. Such adsorption ability for each biopolymer correlates with the final crystallinity of the crosslinked compound. The chemically modified chitosan ChEp presented the lowest adsorption capacity, due to the effectiveness of this agent in crosslinking chitosan, to give rigidity to the final structure, as a consequence of its short chain length. As expected, after deprotonation the adsorption capacity of the glutaraldehyde derivative was enhanced, indicating that the crosslinking reaction is effective in protecting the precursor biopolymer under acidic conditions and it is an efficient way to preserve the amino function on the polymeric structure recuperated, after proton removal. This established procedure guarantees identical adsorption properties for a rigid three-dimensional network structure. The interactions between the cation and the basic centers at the solid/liquid interface followed by calorimetric titrations showed favorable thermodynamic values, suggesting the possibility of application of these biomaterials for copper removal from wastewater of industrial effluents.

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