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Thermodynamic Study of the Protonation and Interaction with Metal Cations of Three Chitin Derivatives

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ABSTRACT

The chelating ability of N-carboxymethyl chitosan, chitosan and fully deacetylated chitosan was assessed for dilute solutions of Cu and Pb by means of potentiometry, calorimetry, dilatometry and circular dichroism measurements N-Carboxymethyl chitosan, to which a significant freedom of the glycine residues imparts low conformational rigidity, was much more effective than chitosan and deacetylated chitosan in binding both Cu(II) and Pb(II) Dilatometry gave positive ΔV values for N-carboxymethyl chitosan only, as a consequence of the desolvation of the ionic species, mainly carboxyl groups, in the course of the binding reaction The carboxyl group was not the only site of reaction, however, as indicated by the negative enthalpy variations It is known that the reactions of metal ions with polycarboxylates as a rule are endothermic (positive ΔH) and those with polyamines are exothermic (negative ΔH), it is therefore concluded that the complexing ability of the amino group is corroborated by the presence of the carboxyl group in the glycine residue of N-carboxymethyl chitosan

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INTRODUCTION

While chitosan is amply recognized as a chelating polysaccharide whose nitrogen and oxygen atoms act as electron donors (Muzzarelli, 1973), efforts towards enhancement of its chelating ability have also been made (Muzzarelli *et al*, 1987) The introduction of additional chemical functions in the glucosamine unit is expected to impart higher capacity or selectivity, especially when the nitrogen atom is kept in the form of an amine rather than an amide.

The carboxymethylation of chitosan is a straightforward approach to the introduction of carboxyl groups, and can be mainly achieved either via Schiff reaction with glyoxylic acid leading to *N*-carboxymethyl chitosan (otherwise called glycine glucan when highly substituted; Muzzarelli *et al*, 1982*a*) or by etherification at C-6 with chloroacetic acid, according to Tokura *et al* (1983) A review article has been published on carboxymethylated chitins and chitosans (Muzzarelli *et al*, 1989)

Evidence of the enhanced capacities of the aminoacid-substituted glucans obtained from chitosan for a number of transition metal ions has been published (Muzzarelli *et al*, 1985; Muzzarelli & Zattoni, 1986). That information, collected for insoluble aminoacid glucans, showed that they possess capacities one order of magnitude higher than chitosan under identical conditions and that their capacities are very slightly dependent on metal ion concentration as a further point of difference from chitosan

The purpose of the present work is to obtain a picture of the thermodynamic behaviour of chitosan and its derivatives, in order to characterize the said polysaccharides and to offer an explanation for the chelating capacity enhancement. To achieve this aim, proton dissociation and Cu(II) and Pb(II) binding by chitosan, fully deacetylated chitosan and *N*-carboxymethyl chitosan in dilute aqueous solution were studied.

MATERIALS AND METHODS

Materials

Chitosan from *Euphausia superba* was supplied by Chito-Bios, Ancona, Italy Its degree of deacetylation, as determined by spectrophotometry (Muzzarelli, 1986) was 0·58 It was dissolved in 1% acetic acid to prepare chitosan acetate solutions which were extensively dialysed by using Visking dialysis tubings manufactured by Serva, Heidelberg, with exclusion limits 8000–15 000 daltons (average pore diameter 240 nm).

The water-soluble *N*-carboxymethyl chitosan was prepared according to the procedure described by Muzzarelli *et al* (1982*b*), by using glyoxylic acid and reducing the corresponding aldimine *N*-Carboxymethyl chitosan, as a viscous solution or a gel, was added to 0 01 M acetic acid (the pH of the dialysed solution was never lower than 3), filtered, exhaustively dialysed against water and then freeze-dried The average molecular weights of chitosan and *N*-carboxymethyl chitosan were 465 000 and 543 000 daltons, respectively (Muzzarelli *et al*, 1987) Deacetylated chitosan with degree of deacetylation higher than 0 97 was prepared as already described (Muzzarelli, 1973). A sample of C6-oxycellulose was also employed for comparison purposes. It was prepared following the procedure outlined by Painter (1977) Cellulose was dissolved in 85% (w/w) orthophosphoric acid and then oxidized upon addition of anhydrous sodium nitrite as a fine powder Further details are to be found elsewhere (Cesàro *et al*, 1985, Painter *et al*, 1985) The sample was stored as a freeze-dried solid in Na form The viscometric molecular weight was 12 000 daltons Details of the physico-chemical characterization of the product were published by Cesàro *et al* (1987)

Cu and Pb perchlorates were Ventron GmbH products Their aqueous solutions were titrated with EDTA All the other chemicals employed were Carlo Erba analytical grade products Deionized, doubly distilled water was used for dialysis. Measurements were done on freshly prepared solutions

Preparation of polymer solutions

Chitosan and deacetylated chitosan solutions were prepared by dissolving a calculated amount of the freeze-dried polymer in an excess of dilute acetic acid (pH ~3). The solutions were used after filtration and exhaustive dialysis against water The polymer concentration was computed from the weight of the polymer and the final volume of the solution. The amount of water in the solid polysaccharide was determined with a Mettler DL 18 apparatus, employing the Karl–Fisher method The amount of water varied from 15% to 21% in the samples obtained from various preparations

N-Carboxymethyl chitosan solutions were prepared by dissolving the freeze-dried material in very dilute acetic acid The solution obtained was filtered and then dialysed against water to remove excess acid The concentration was evaluated based on the amount of dissolved polysaccharide and the volume of the final solution, and confirmed by potentiometric titration under nitrogen

Solutions of oxycellulose were freshly prepared from the solid by dissolution in water. The titer of the polymer solution was determined four times after dialysis against 0·1 M aqueous acetic acid, and against water, until disappearance of acetic acid in the outer compartment of the dialysis system. The cloudy solution of C6-oxycellulose was homogenized and an aliquot was titrated while another aliquot was neutralized by addition of known amounts of NaOH.

Instruments and methods

The potentiometric titrations were performed with a Radiometer PHM52 pH meter, equipped with GK 2321C combined electrodes.

The viscometric determinations were made with an AVS/G Schott-Gerate automatic apparatus, employing Ubbelohde viscometers. The measurements were made on solutions prepared by dissolving the polymer in HCl (*N*-carboxymethyl chitosan) or in acetic acid (chitosan). The correct ionic strength, I, was reached by addition of NaCl. In the evaluation of I the concentration of all the ionic species in solution, including the polyelectrolyte itself, were taken into account.

The calorimetric measurements were performed using a LKB 10700-2 batch type twin calorimeter, equipped with gold cells, employing standard procedures. In each protonation experiment, 2 ml of polymer solution ($C_p = 4{\cdot}8 \times 10^{-3}$ M) and a calculated amount of 5×10^{-4} M $HClO_4$ solution were put in the reaction cell, while in the reference cell an equal volume of water replaced the $HClO_4$ solution. Therefore, the heat read was already corrected for the polymer dilution enthalpy (the heat of dilution of $HClO_4$ being very small and hence negligible) With *N*-carboxymethyl chitosan the volumes of $HClO_4$ were chosen to have a variation of degree of dissociation (α) in a single protonation experiment ranging from 0·03 to 0·12; with both chitosan and deacetylated chitosan the variation of α was ~0·6. The amount of bound protons was computed by potentiometric determination before and after the calorimetric reaction. In the interaction with Cu(II) and Pb(II), the acid solution was replaced with calculated small amounts of a perchlorate solution (concentration of the cation, $2\text{–}4 \times 10^{-2}$ M).

The dilatometric determinations were performed with Carlsberg dilatometers, immersed in a high-performance thermostatic bath (Delben, 1980). The experimental details were reported by Crescenzi *et al.* (1974). The solutions were introduced into the dilatometers with the aid of a glass Hamilton syringe with a teflon plug.

The dichroic spectra were recorded from 320 to 200 nm with a Jasco J500-A dichrograph, equipped with a Jasco DP500-N data processor. Four spectra were currently combined for each determination.

From the potentiometric titrations, the values of the negative logarithm of the dissociation constant, pK_a, were calculated as a function of α. The latter was computed from the amount of dissociating groups on the polyelectrolyte, which corresponds to the amount of deacetylated sugar units in the polymer backbone, by the expression

$$\alpha = \bar{\alpha} + \frac{[H^+]}{C'_p} - \frac{[OH^-]}{C'_p}$$

were $\bar{\alpha}$ is the degree of neutralization (amount of added base versus the amount of base required to neutralize the acidic groups fully) and C'_p is the equivalent concentration of the polysaccharide. The degree of deacetylation of the samples being 0·58, $C'_p = 0{\cdot}58\,C_p$, where C_p is expressed in moles of total saccharide units per dm^3. In the case of *N*-carboxymethyl chitosan, for which the pK_a versus α plots are reported, the limits of α are 0 and 2, owing to its doubly ionizable aminoacid side groups

For *N*-carboxymethyl chitosan, the pK_a values were obtained in the range $0 > \alpha > 1$, employing the expression derived by Dubin & Strauss (1970)

$$pK_a = pH + \log\left\{\frac{1}{2}\left(\frac{1-\alpha}{\alpha}\right) + \frac{1}{2}\left[\left(\frac{1-\alpha}{\alpha}\right)^2 + \frac{4K°(2)}{K°(1)}\left(\frac{2-\alpha}{\alpha}\right)\right]^{1/2}\right\}$$

where $K°(1)$ and $K°(2)$ are the 'intrinsic' first and second ionization constants, respectively In the range $1 < \alpha < 2$, the pK_a values were calculated by using the approximate equation

$$pK_a = pH + \log\frac{2-\alpha}{\alpha-1}$$

RESULTS AND DISCUSSION

Interaction with protons and structural considerations

In Fig. 1 the titration curves of *N*-carboxymethyl chitosan are reported for two polymer concentrations, C_p; in each of them two inflection points are evident and correspond to the dissociation of two chemically different groups.

The trend of pK_a versus α, reported in Fig 2, is typical for a polyelectrolyte bearing two different and independent sets of dissociating

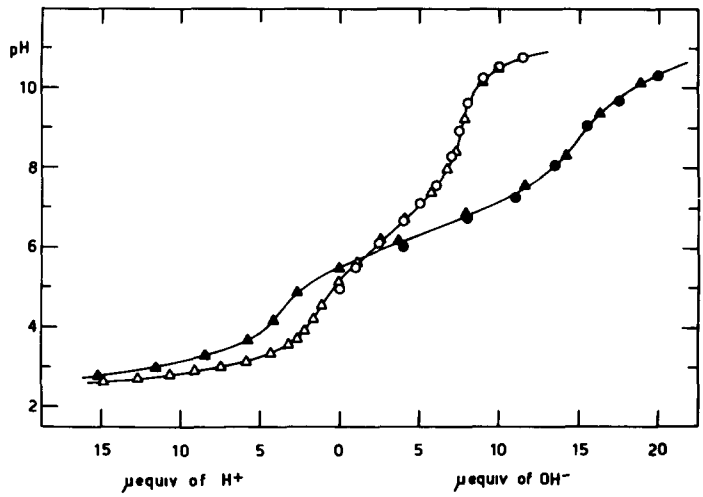

Fig. 1. Potentiometric titration curves of *N*-carboxymethyl chitosan in water at 25°C ○△, initial polymer concentration, $C_{p(i)}$, 2.1×10^{-3} M, ●▲, $C_{p(i)} = 4.8 \times 10^{-3}$ M, ○●, addition of NaOH, △▲, addition of $HClO_4$ to the fully dissociated polymer

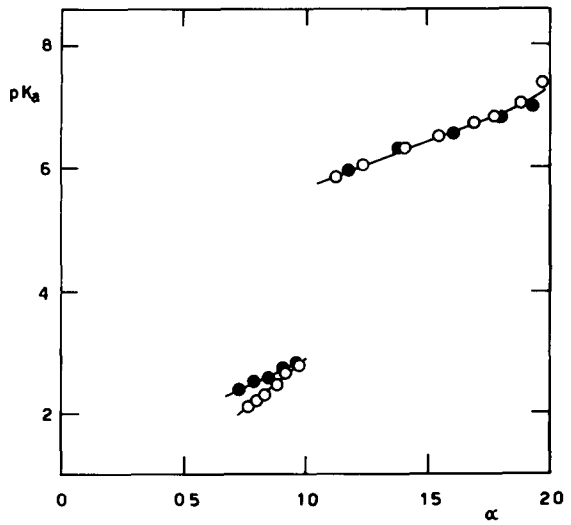

Fig. 2. Plot of pK_a versus the degree of dissociation, α, for *N*-carboxymethyl chitosan in water at 25°C ○, initial polymer concentration, $C_{p(i)}$, 2.0×10^{-3} M, ●, $C_{p(i)} = 4.8 \times 10^{-3}$ M

groups (Bianchi *et al*, 1970, Dubin & Strauss, 1970). The constancy of pK_a at varying C_p values may be due to the considerable rigidity of the polymer chain which, although not so pronounced as in the case of the parent chitosan (see below), prevents big differences in the conformation

of the polymer chains, which should remain in an expanded conformation, regardless of the pH The small differences shown in Fig. 2 appear to be due to the experimental uncertainty, which is evaluated to be at least $\pm 0{\cdot}1$ pK_a unit at the lowest values explored.

By using the pK_a values of Fig 2 and employing the popular stoichiometric equations, we computed the relative amounts of the fully protonated, monodissociated, and fully dissociated forms as a function of pH The results are reported in Fig. 3 Although approximate (e g. the minor correction for the ionic strength was omitted), the results of Fig 3 depict the actual average situation on the ionizable groups of *N*-carboxymethyl chitosan as a function of pH, for instance, at pH ~6, α is ~1 3, which means that one-third of the groups are fully dissociated, and two-thirds are monodissociated This may be of interest in certain cases, such as the binding of transition and post-transition metal ions, which is discussed below and, in more detail, by Delben & Muzzarelli (1989)

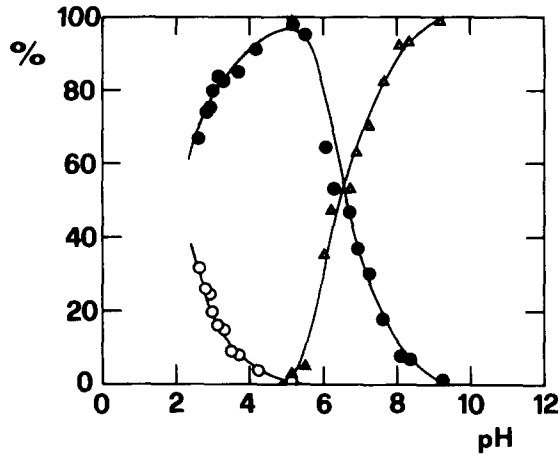

Fig. 3. Relative amount (expressed as,%) of the fully protonated form (○), monodissociated form (●) and fully dissociated form (△) for *N*-carboxymethyl chitosan in water at 25°C as a function of the pH value, initial polymer concentration $4\,8 \times 10^{-3}$ M

Evidence of a rigid conformation of both chitosan and *N*-carboxymethyl chitosan in solution was supplied by circular dichroism measurements Unlike fully deacetylated chitosan, both *N*-carboxymethyl chitosan and chitosan show a deep negative CD band centered around 208 nm, attributed to $n \rightarrow \pi^*$ electron transition of the chromophore -NH-CO (Kabat *et al.*, 1969, Stone, 1969, Dickinson & Bush, 1975) The shape of this band is in agreement with that reported by Domard (1986) and other authors there quoted We found that this

band was not affected by varying the pH of the solution from 2 to 11. The same independence of the CD spectra was found by Domard (1986) for NAcGlc oligomers. The CD spectra are not included here for the sake of simplicity. These findings seem to confirm that the ionizable side groups of *N*-carboxymethyl chitosan should be quite independent of the neighbouring atoms in the polymer chain.

In this context, the calorimetric and the dilatometric results appear not to have a simple interpretation. The calorimetric results for the protonation, reported in Table 1, show no difference in the ΔH of

TABLE 1
Calorimetric Data of Protonation

Polymer	*Range of* α	$-\Delta H_p$ *(±SD) (kcal/mole of bound proton)*
N-Carboxymethyl chitosan	0–1	5 1±0 2
	1–2	7 5±0 6
Deacetylated chitosan	0–1	2 6±0 3
Chitosan	0–1	7 2±0 3

protonation (ΔH_p) in going from chitosan to *N*-carboxymethyl chitosan ($1 < \alpha < 2$), notwithstanding the large chemical difference in the group that undergoes protonation. The fact that in the case of *N*-carboxymethyl chitosan the ΔH_p values of both protonable groups are similar, in spite of predictions by current theories on polyelectrolytes (Manning, 1969; Paoletti *et al*, 1985), is also surprising. The value found ($1 < \alpha < 2$) is close to the ΔH_p for the anion of glycine (Datta & Grzybowski, 1958) and very close to the corresponding values for dimethylglycine (Datta & Grzybowski, 1958) and for *N*-tris(hydroxymethyl)methane glycine (Bates *et al*, 1974). The constancy of ΔH_p found for *N*-carboxymethyl chitosan as a function of α is easier to explain In fact, the above findings confirm other evidence, i.e the dependence of the polymer chain rigidity on α and, moreover, a substantial freedom of the reactive centres of the polymer backbone.

On the other hand, the experimental values of ΔV associated with the proton coupling for the three polysaccharides studied is practically zero (for *N*-carboxymethyl chitosan this value was found for both dissociation steps). If compared with the data for synthetic and natural polycarboxylates (Crescenzi *et al*, 1974; Delben *et al*, 1974; Fenyo *et al*, 1977; Delben & Crescenzi, 1978, Paoletti *et al*, 1981; Cesàro *et al*,

1982, 1987), this result is unexpected indeed, at least for *N*-carboxymethyl chitosan. Because in the protonation of the carboxyl group a large amount of electrostricted water is liberated (Crescenzi *et al*, 1974), with a large increase of the solution volume, a possible explanation for our findings would be that only the amino groups of *N*-carboxymethyl chitosan are protonated, at low pH values this is against every reasonable prediction and the potentiometric evidence

The viscometric evidence provides further support to the above mentioned rigidity of the polymer chain for chitosan and its derivatives. In Fig. 4, the intrinsic viscosity, $[\eta]$, is reported versus I, for the three polysaccharides under study and for C6-oxycellulose. The latter was chosen to compare the stiffness of chitosan and its derivatives with that of another polysaccharide formally derived from the same anhydroglucosidic chain. The viscometric results are summarized in Table 2.

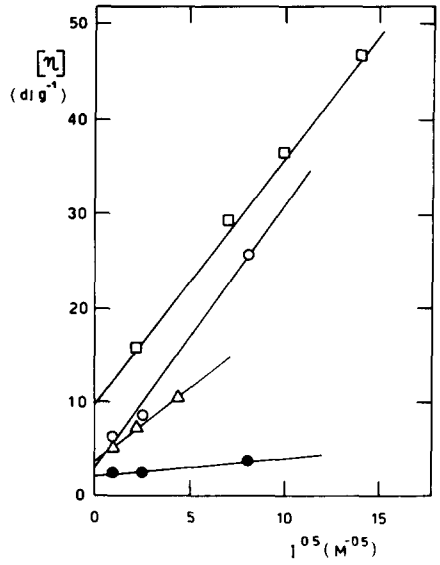

Fig. 4. Plot of the intrinsic viscosity, $[\eta]$, versus $1/\sqrt{I}$ (where I is the ionic strength of the solution) for chitosan (□), deacetylated chitosan (○), *N*-carboxymethyl chitosan (△) and C6-oxycellulose (●)

From the Smidsrød parameter, which is empirically derived from the slope of the plots in Fig. 4 and the value of $[\eta]$ at $I = 0{\cdot}1$ M (Smidsrød & Haug, 1971), the following trend for the rigidity of the polymer chain is obtained: chitosan > C6-oxycellulose > *N*-carboxymethyl chitosan > deacetylated chitosan. Notwithstanding the large uncertainty of this method, this trend appears to be significant but not simple to interpret. It

TABLE 2
Viscometric Data

Polymer	*Degree of substitution*	S^a	$[\eta]_{0.1}$ (dl/g)	B^b
N-Carboxymethyl chitosan	58[c]	1 555	8 58	0 095
Deacetylated chitosan	3[c]	2 795	11 45	0 117
Chitosan	58[c]	2 592	18 45	0 059
C6-Oxycellulose	95[d]	0 242	2 71	0 066

[a] S is the slope of $[\eta]$ versus $I^{-1/2}$, where I is the ionic strength of the solution
[b] B is the Smidsrød stiffness parameter
[c] Degree of deacetylation
[d] Degree of oxidation at C6

is clear that the substituent in position 2 plays an important role in establishing a rigid conformation of the polysaccharidic chain, as theoretically postulated by Terbojevich *et al* (1986). The lower conformational rigidity of *N*-carboxymethyl chitosan stems from a significant freedom of the short side-chains bearing the carboxyl groups (glycine residues), probably because they are relatively away from the polymer chain.

Interactions with Cu(II) and Pb(II)

The interactions of Cu(II) and Pb(II) with chitosan, deacetylated chitosan and *N*-carboxymethyl chitosan were studied by potentiometry, microcalorimetry, dilatometry, spectropolarimetry and UV absorption spectroscopy. The results obtained with the two latter techniques are reported and discussed in the paper by Delben & Muzzarelli (1989)

The potentiometric and calorimetric results are reported in Figs 5 and 6, respectively. In all cases, the thermodynamic variable considered is reported against R, the molar ratio of the added metal counterion concentration to C_p. As for the potentiometric results, the usual interpretation is that the pH decrease is a consequence of the binding of countercations by the polymer. As a consequence, the larger affinity of the polysaccharide for a particular cation with respect to the proton is directly read from the pH extent of the pH decrease. From the data in Fig. 5, it appears that *N*-carboxymethyl chitosan is much more effective in binding Cu(II) and Pb(II) than the parent chitosan. The fully deacetylated chitosan is even less effective than plain chitosan. The picture is well paralleled by the calorimetric evidence in Fig. 6. In principle the enthalpy change is not necessarily proportional to the amount of bound

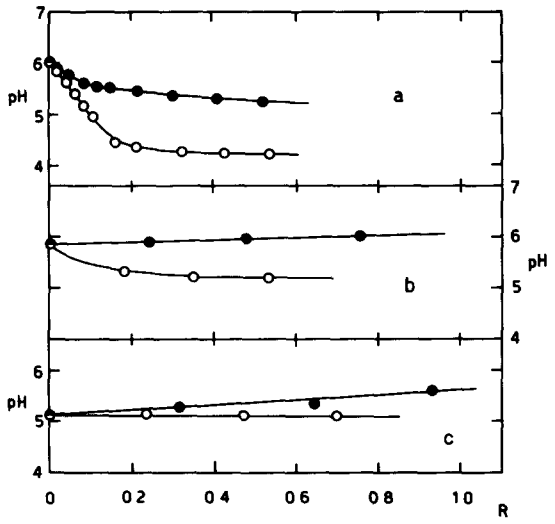

Fig. 5. Variation of pH on addition of divalent cations to (a) *N*-carboxymethyl chitosan, (b) chitosan and (c) deacetylated chitosan, in water at 25°C ○, Cu(II), ●, Pb(II)

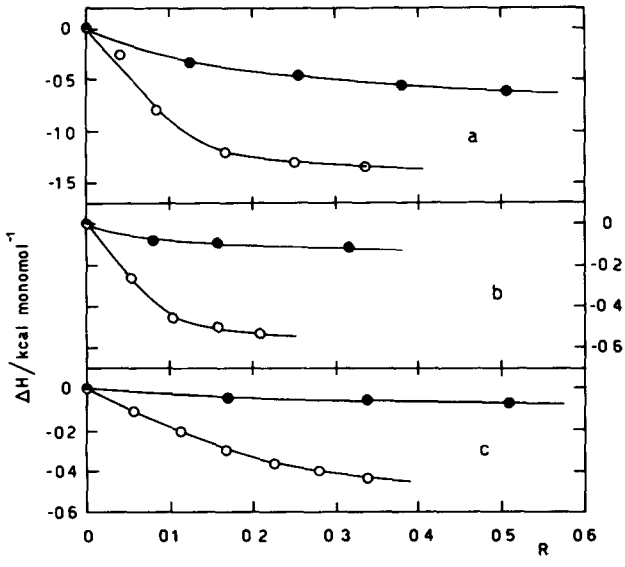

Fig. 6. Enthalpy change on addition of divalent cations to (a) *N*-carboxymethyl chitosan, (b) chitosan and (c) deacetylated chitosan, in water at 25°C ○, Cu(II), ●, Pb(II)

counterions; in fact, it depends on the extent of binding and the nature of the reacting species. However, the complete agreement between the trends indicated by the potentiometric and calorimetric data seems to indicate that the site of binding in the three polysaccharides considered

should be essentially the same, and hence the evaluation of the relative binding ability is allowed. The partially acetylated chitosan appears to be a better chelating agent than the fully deacetylated chitosan; the carboxyl groups contribute very effectively to the binding ability of the polysaccharide.

This view is also supported by the dilatometric results, summarized in Table 3, and the dichroic spectra (Delben & Muzzarelli, 1989) The dilatometric data show that positive ΔV are found only for Cu(II) and Pb(II) reacting wth *N*-carboxymethyl chitosan. This is interpreted in terms of desolvation of the interacting ionic species in the course of the binding reaction. The ΔV values being zero for both chitosan and fully deacetylated chitosan, the difference seems ascribable to the effect of the carboxyl group but it should not be considered the only site of interaction between the countercations and the polymer.

TABLE 3
Dilatometric Data

Polymer	*Interacting ion*	R^a	ΔV *(ml/mole of bound ion)*
N-Carboxymethyl chitosan	H^+	1 035	0 8
	Cu^{2+}	0 271	40 2
	Pb^{2+}	0 296	26 0
Deacetylated chitosan	H^+	2 058	0
	Cu^{2+}	0 108	0
	Pb^{2+}	0 162	0
Chitosan	H^+	0 959	−3 5
	Cu^{2+}	0 100	0
	Pb^{2+}	0 151	0

[a]Maximum value of the ratio of interacting ion to polymer molar concentration in the dilatometric experiments

This interpretation is supported by the negative enthalpy variation When the interaction between Cu(II) and polymeric species (Crescenzi *et al.*, 1974; Paoletti *et al.*, 1981; Cesàro *et al.*, 1988) or monomers (McAuley *et al*, 1967, Aruga, 1981) in aqueous solution is performed only or mainly by carboxyl groups, as a rule the reaction is endothermic, i.e. characterized by a positive ΔH value which indicates that the binding process is driven by a large increase of entropy due to a large amount of water liberated during the binding. Negative ΔH values for exothermic reactions of polycarboxylates with Cu(II) are also known, but it was

suggested that a conformational transition of the polymer chain accompanies the polyion–counterion interaction (Cesàro *et al*, 1982) On the other hand, exothermic reactions in aqueous solution between Cu(II) and amino groups lodged on oligomers or on polymers have been reported (Barbucci *et al*, 1983), they seem to represent the rule for those systems Moreover, the ΔV values relevant to Cu(II) and Pb(II) are positive only in the case of *N*-carboxymethyl chitosan, while they are zero for the chitosans.

CONCLUSIONS

Based on the thermodynamic findings reported, we can conclude that in these homogeneous systems the counterions are bound by nitrogen (presumably with contributions of the hydroxyl groups), with an important contribution of the carboxyl groups in the case of *N*-carboxymethyl chitosan. From the quantitative standpoint, the data here discussed indicate that the chelating ability of the modified chitosans is in the order. *N*-carboxymethyl chitosan > chitosan > deacetylated chitosan Data show that, while Cu(II) is bound by all three of the polysaccharides to different extents, Pb(II) is bound mostly by *N*-carboxymethyl chitosan The difference of affinity between amino groups on the one hand and Cu(II) or Pb(II) on the other hand is also to be taken into account The present work adds the necessary thermodynamic information to the ample analytical information already published on the subject of metal ion chelation by chitosan and its derivatives

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