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Thermodynamic Study of the Interaction of *N*-Carboxymethyl Chitosan with Divalent Metal Ions

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ABSTRACT

Potentiometry and microcalorimetry indicate that calcium is not chelated by N-carboxymethyl chitosan and that the order of affinity of N-carboxymethyl chitosan for the other divalent metal ions studied is $Cu > Cd \gg Pb$, $Ni > Co$ *For these metals the following binding constants were obtained, respectively* 7×10^5, 4×10^5, 3×10^3, 3×10^3 *and* 1×10^3 *The number of water molecules liberated in the binding process by the solvated metal ions and by carboxylate were 25, 14 and 16 for Cu(II), Cd(II) and Pb(II), respectively The binding was enthalpy driven and the negative* ΔH *values were thus attributed to the interaction of metal ions with nitrogen atoms Cu(II) and Pb(II) introduced new bands in the CD and UV spectra, whilst the other metal ions did not for Cu(II) they were a positive band at 237 nm and a negative one at 277 nm in the CD spectrum, and an absorption band at 246 nm in the UV spectrum, for Pb(II) they were a positive band at 229 nm and a negative one at 244 nm in the CD spectrum, and an absorption band at 231 nm in the UV spectrum*

INTRODUCTION

The relative ability of *N*-carboxymethyl chitosan, chitosan and fully deacetylated chitosan in binding Cu(II) and Pb(II) ions in dilute aqueous solution was discussed by Delben *et al* (1989), who suggested an

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interpretation of the different binding abilities in terms of complex formation by the substituting group present on C2 of the polysaccharides studied.

In the present work, attention is focused on *N*-carboxymethyl chitosan which was found to exhibit much higher chelating ability than chitosan. The collection of more complete data on the chelation process in homogeneous solutions, a better understanding of the binding reaction and a wider picture of the behavior of *N*-carboxymethyl chitosan towards a number of divalent metal ions are within the scope of this article

Data on the binding capacity of some aminoacid-substituted glucans obtained from chitosan for a number of transition metal ions have been published by Muzzarelli *et al.* (1985) and by Muzzarelli & Zattoni (1986), that information was however mainly in terms of chelation isotherms for insoluble substituted chitosans, whilst the object of the present study is the binding ability of *N*-carboxymethyl chitosan (also called glycine glucan) as a soluble compound in dilute solutions, where weak or negligible interactions between the polymer chains occur.

MATERIALS AND METHODS

Materials

The water-soluble *N*-carboxymethyl chitosan was prepared according to the procedure described by Muzzarelli *et al* (1982) from *Euphausia superba* chitosan (degree of deacetylation 58%) supplied by Chito-Bios, Ancona, Italy. Its average molecular weight was 543 000 (Muzzarelli *et al*, 1987; Muzzarelli, 1988). Other materials were the same as indicated earlier (Delben *et al*, 1989). Perchlorate salts were preferred in view of the very low tendency of perchlorate anion to form ionic pairs even in concentrated solutions; other important problems caused by other anions are avoided when using perchlorates. For instance, chloride absorbs in the UV region and is able to form complexes with transition metal cations, nitrate may act as an oxidant and many sulphates are slightly soluble

Instruments and methods

The dilatometric measurements were performed with Carlsberg dilatometers, following a procedure similar to that described by Rasper & Kauzmann (1962), the only difference being the use of *n*-heptane instead of kerosene. *n*-Heptane (Carlo Erba) was purified as described by Katz & Ferris (1966). The dilatometers were immersed in a high-performance thermostatic bath (Delben, 1980). In each set of experiments, 3–4 ml of perchlorate solution and the same volume of *N*-carboxymethyl chitosan solution of known concentrations were kept in the two arms of the dilatometers until the desired temperature was reached. The solutions in the two arms were then mixed and the volume changes recorded based on the positions of the *n*-heptane meniscus before and after mixing. Volume changes due to the dilution of both solutions to be mixed were also measured and corrections for these values were made. The accuracy of the method is discussed elsewhere (Delben, 1980, Delben *et al*, 1982)

The UV absorption data were collected with a Varian Cary 2200 spectrophotometer Atomic absorption measurements were performed with a Perkin-Elmer 300 spectrometer Circular dichroism and UV absorption measurements were made after introducing the correct amount of metal perchlorate solution directly into the cuvette containing the desired volume of the polymer solution, with the aid of a Hamilton precision microsyringe. Absorption spectra were recorded as differences between polymer-containing and polymer-free solutions, after thorough stirring. No kinetic phenomena were observed. Other instruments and methods were those indicated previously (Delben *et al*, 1989)

In the thermodynamic experiments, C_p, the molar polymer concentration (moles of total polysaccharide units per dm^3), was $4\text{–}7 \times 10^{-3}$, in the UV absorption and CD measurements, C_p was 10 times lower. The pH of the polymer solutions was 5·2–6·0, depending on C_p Freshly prepared solutions were employed throughout

RESULTS AND DISCUSSION

The whole set of results obtained by means of potentiometry and microcalorimetry is reported in Figs 1 and 2, respectively. The thermodynamic quantity change measured upon mixing dilute *N*-carboxymethyl chitosan solutions with various salt solutions to reach the final ion-to-polymer molar ratio, *R*, is plotted against *R*. With both of the techniques employed, the maximum effect is shown by Cu(II), which can be supposed to be the most extensively bound ion. In contrast, Ca(II) appears not to be bound at all, or in any case beyond the detection limits of the techniques here employed From the results here reported, the order of affinity of *N*-carboxymethyl chitosan for the divalent cations considered is $\mathrm{Cu} > \mathrm{Cd} \gg \mathrm{Pb}, \mathrm{Ni} \geqslant \mathrm{Co} \gg \mathrm{Ca}$.

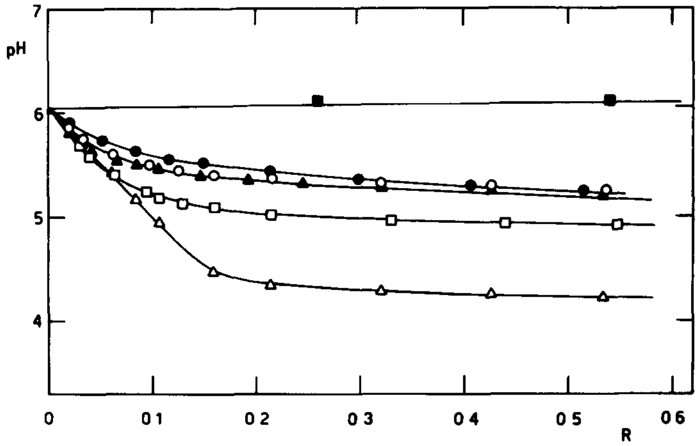

Fig. 1. Variation of pH on addition of divalent cations to *N*-carboxymethyl chitosan in water at 25°C ■, Ca(II), ●, Pb(II), ○, Co(II), ▲, Cd(II), □, Ni(II), △, Cu(II), *R* is the molar ratio of cations added as perchlorates to total saccharidic units

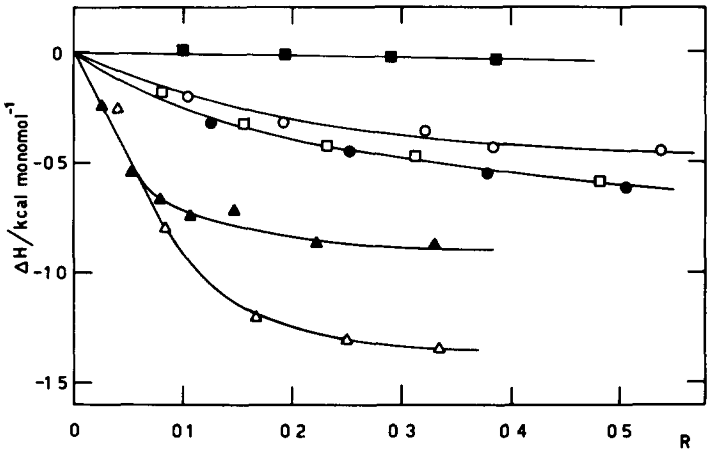

Fig. 2. Enthalpy change on addition of divalent cations to *N*-carboxymethyl chitosan in water at 25°C ■, Ca(II), ○, Co(II), □, Ni(II), ●, Pb(II), ▲, Cd(II), △, Cu(II)

The surprisingly low or zero affinity of Ca(II) ions for *N*-carboxymethyl chitosan prompted us to verify the effects of the same ions on the parent chitosan. Again a zero effect was also found (up to $R = 0{\cdot}5$, experimental data not reported), in agreement with early data obtained by Muzzarelli (1973). We have verified the presence of Ca(II) in the solutions, because chitosan may contain calcium salts, detectable in its ashes. Chitosan and *N*-carboxymethyl chitosan solutions were analysed by atomic absorption spectrometry and no more than 0·23 mg Ca/liter was found in the solutions, corresponding to $R \leqslant 0{\cdot}0015$ under our experimental conditions, thus confirming the validity of our results

Two additional conclusions can be drawn from the data in Figs 1 and 2 Firstly, Cu(II) seems to saturate *N*-carboxymethyl chitosan, while all the other divalent cations are unable to reach saturation, the thermodynamic quantity still decresaing even at the extreme values of R explored The R value at saturation being 0 12, on the hypothesis that Cu(II) is mainly bound by *N*-carboxymethyl residues, it is estimated that at saturation each copper ion is surrounded by four *N*-carboxymethyl residues.

Secondly, from the calorimetric curves a quantitative evaluation of the amount of counterion bound can be tentatively made, with the aid of the well known Scatchard method. When a linear dependence of $1/\Delta H$ on $1/R$ is verified, as in our case, it is possible to obtain the values of ΔH at R equal to infinity The values of the binding constants so computed are reported in Table 1, together with the analogous quantities derived from the UV and CD results (see below) Calculations were made assuming a single site of binding The value of n, the ratio of moles of bound counterions to polymer units at saturation, in the cases considered, ranged from 0 08 to 0·13 (see Table 1)

TABLE 1

Thermodynamic Data for Binding of Divalent Metal Ions by *N*-Carboxymethyl Chitosan in Water at 25°C

Counterion	n^a	K *(liters/mol)*	*Technique and λ*	ΔG^b *(kcal/mol)*	ΔH^b *(kcal/mol)*	ΔS *(e u)*	ΔV *(ml/mol)*	w^c
Copper(II)	0 11	$9\ 2 \times 10^5$	CD, 237 nm	−8 1	−9 4(±0 2)	−4 4	40 2	25
	0 11	$5\ 4 \times 10^5$	CD, 276 nm	−7 8		−5 3		
	0 11	$6\ 3 \times 10^5$	UV, 246 nm	−7 9		−5 0		
	0 11	$7\ 8 \times 10^5$	ΔH	−8 0		−4 6		
Lead(II)	0 12	$4\ 9 \times 10^3$	CD, 244 nm	−5 0	−6 9(±0 5)	−6 4	26 0	16
	0 12	$2\ 7 \times 10^3$	UV, 234 nm	−4 7		−7 4		
	0 12	$2\ 2 \times 10^3$	ΔH	−4 6		−7 9		
Cobalt(II)	0 13	$1\ 0 \times 10^3$	ΔH	−4 1	−5 1(±0 3)	−3 4	(45)	(28)
Nickel(II)	0 12	$2\ 6 \times 10^3$	ΔH	−4 7	−6 9(±0 5)	−7 6	(56)	(35)
Cadmium(II)	0 08	$4\ 0 \times 10^5$	ΔH	−7 6	−9 4(±0 2)	−5 9	23 6	14

Experimental uncertainties are shown in parentheses

[a] Ratio of moles of bound metal ions to polymer repeating units at saturation

[b] Expressed as kcal/mol of bound counterions

[c] Number of water molecules liberated per bound counterion

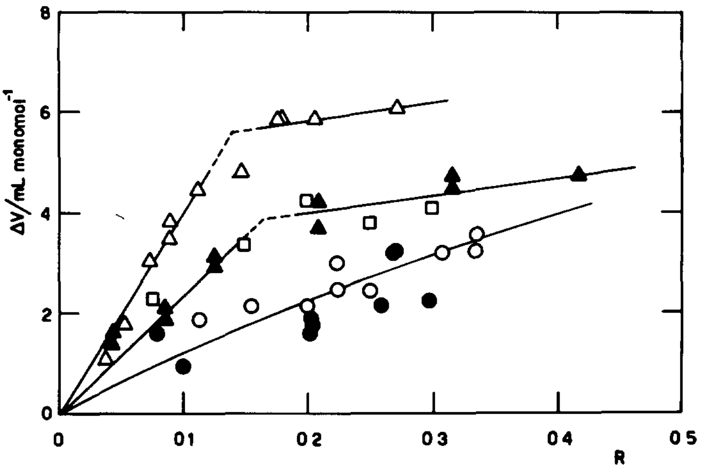

Fig. 3. Volume increase on addition of divalent cations to *N*-carboxymethyl chitosan in water at 25°C △, Cu(II), □, Ni(II), ▲, Cd(II), ○, Co(II), ●, Pb(II)

Keeping these results in mind, one can try to interpret the dilatometric data reported in Fig. 3. First, this figure presents nearly the same general features already discussed. Moreover, the affinity constant of Cu(II) for *N*-carboxymethyl chitosan being appreciably high, from the plot in Fig. 3 a calculation can be made for the system *N*-carboxymethyl chitosan–Cu(II) of the volume variation associated with the binding of copper by the polysaccharide chain (which corresponds to the slope of the extreme left part of the plot in Fig. 3). The result is 40 ml per mole of bound Cu(II). From this value, with the aid of the expression

$$w = \frac{\Delta V}{18(1/d^\circ - 1/d)}$$

(where d° and d are respectively the density of the free water and of the electrostricted water in the hydration spheres), assuming that $d = 1.1$ g/cm^3 as found in ultrasound absorption measurements (Yasunaga & Sazaki, 1951), one can calculate w, the number of water molecules liberated in the binding process. The value found, 25 per each ion bound, appears to be due to the dehydration of copper ions and of carboxyl groups, engaged in the binding process. While values of similar order of magnitude were normally found when studying the interactions of synthetic and natural polycarboxylate–Cu(II) systems (Crescenzi *et al.*, 1974, Delben *et al*, 1974, Paoletti *et al*, 1981, Cesàro *et al.*, 1988*b*), for chitosan at different degrees of deacetylation (where carboxyl groups are absent) the ΔV found (and hence w) was zero (Delben *et al.*, 1989).

Similar calculations can be made for the other systems studied, although with more approximate results owing to the comparatively lower values of the binding constants The whole set of computed values appears in the last column of Table 1, where the values of the binding differential thermodynamic functions are also reported The ΔH values correspond to the slopes of the calorimetric curves for $R \rightarrow 0$ They were obtained by using the values of n and of the binding constants reported in Table 1.

Turning attention to the entropy changes, the values found are in all cases negative and very small This, of course, parallels the calorimetric results in the sense that the binding phenomenon in the systems studied is enthalpy driven, the negative ΔH values being very likely due to the interaction of the metal ions with the nitrogen atoms of the polymer (Delben *et al*, 1989). Since the ΔS values are in most cases zero or negative in spite of the positive ΔV values (due to a large w value), the binding should be accompanied by a weak further ordering of the stiff polymer chains. In fact, the negative contribution to total ΔS by the counterions bound by the polymer should be less important because the ratio of bound ions to water molecules liberated is low in each case considered

The binding was also studied by CD and UV spectrophotometry While all the other cations considered did not modify the CD and UV spectra of *N*-carboxymethyl chitosan, Cu(II) and Pb(II) showed interesting features like those found for other natural and synthetic polycarboxylates (Paoletti & Delben, 1975, Manzini *et al*, 1984, Cesàro *et al*, 1988*a*, *b*) The CD spectra of *N*-carboxymethyl chitosan–Cu(II) and *N*-carboxymethyl chitosan–Pb(II) systems are reported in Figs 4 and 5, respectively The spectra were obtained upon the addition of increasing amounts of metal perchlorate in the R range from 0 to ~ 0 6

Copper ions can induce two new dichroic bands in the CD spectrum of *N*-carboxymethyl chitosan, centered around 237 (positive) and 277 (negative) nm, respectively, with a very sharp isodichroic point In addition to this, further evidence of a strong interaction between the polysaccharide chain and Cu(II), the band attributed to the $n \rightarrow \pi^*$ electron transition of the chromophore –NH–CO (Kabat *et al*, 1969, Stone, 1969, Dickinson & Bush, 1975), is only slightly influenced, thus confirming that this group is not the one most involved in the binding process, at least with this polysaccharide and under the experimental conditions chosen. Moreover, this indicates that the conformation of the polymer chain should not be much influenced by the presence of Cu(II) in the binding sites

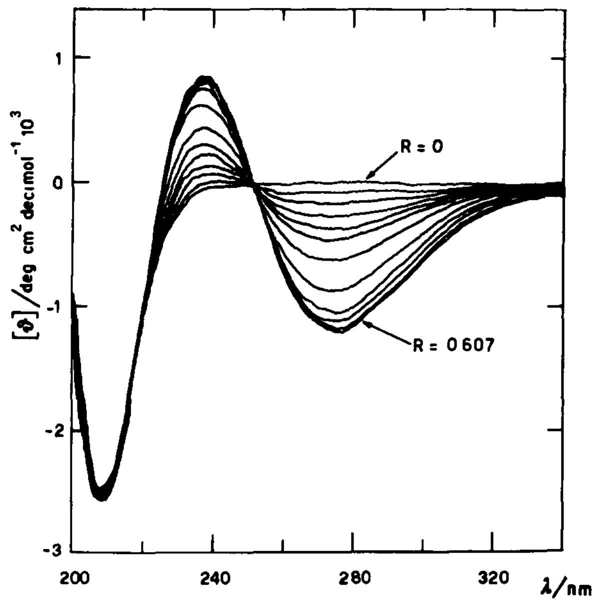

Fig. 4. Circular dichroism spectra of *N*-carboxymethyl chitosan in water on addition of increasing amounts of copper perchlorate, measured as ion-to-polymer molar concentration, *R* Polymer concentration in moles of total saccharidic units × L-1, cell path-length, 1 cm

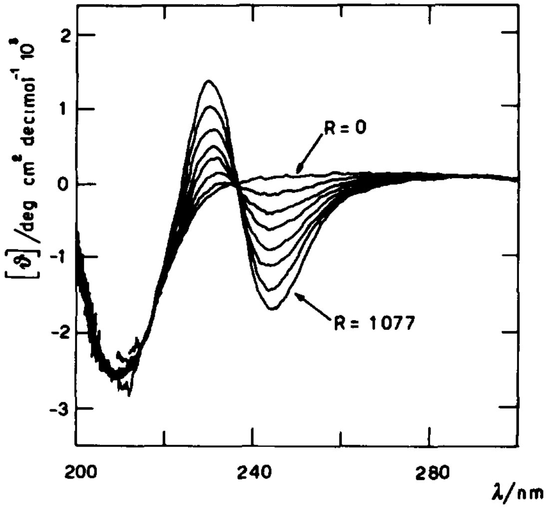

Fig. 5. Circular dichroism spectra of *N*-carboxymethyl chitosan in water on addition of increasing amounts of lead perchlorate, measured as ion-to-polymer molar concentration, *R* Polymer concentration in moles of total saccharidic units × L^{-1}, cell path-length, 1 cm

The CD bands find correspondence in the UV absorption spectra, shown in Fig. 6 The spectra in Fig 6(a), with a very strong band at 246 nm, are very close to those found for similar complexes and interpreted in terms of charge-transfer transitions between the electronic system localized on the ligand (carboxyl group) and the central metal ion (Paoletti & Delben, 1975)

Similar behavior is shown by the *N*-carboxymethyl chitosan–Pb(II) system, as revealed with both CD (Fig. 5) and UV (Fig. 6) measurements As far as the CD spectra are concerned, the main differences between copper and lead are the width of the bands (larger for copper) and the absence of a saturation limit for lead. These findings reveal an easier but more specific binding of copper ions by the polysaccharide, compared with Pb(II). The UV absorption spectra for the *N*-carboxymethyl chitosan–Pb(II) system (Fig 6(b)) are also characterized by a novel absorption band, centered around 234 nm, which is completely distinct from that of the free cation (whose maximum wavelength is 208 nm)

The qualitative picture obtained from the optical and chiro-optical evidence is that the probability of the electronic transition of both Cu(II) and Pb(II) is largely enhanced by the interaction with the polymeric ligand, whose asymmetric binding site is able to induce an evident extrinsic Cotton effect on the electronic transitions of the bound cations

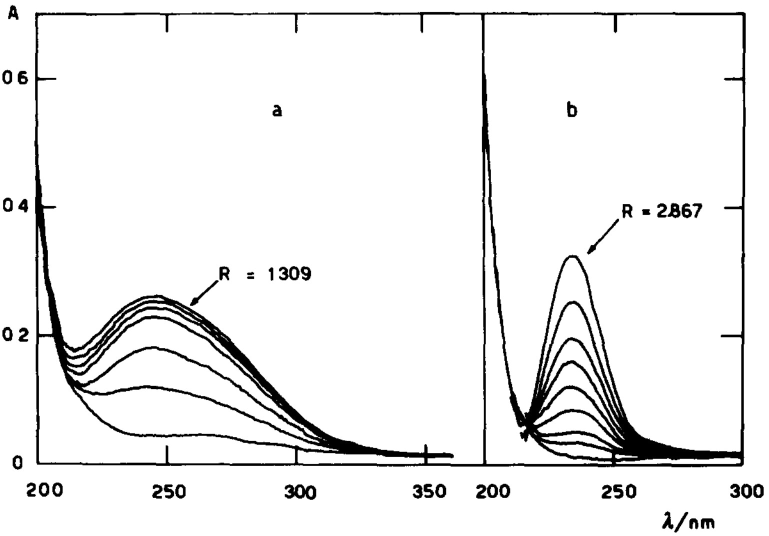

Fig. 6. Ultraviolet absorption spectra of *N*-carboxymethyl chitosan in water on addition of increasing amounts of (a) copper and (b) lead perchlorate, measured as ion-to-polymer molar concentration, *R* Polymer concentration, 2 73 × 10^{-4} and 4 12 × 10^{-4} M in (a) and (b) respectively, cell path-length, 1 cm

It is worth recalling that this behavior is not followed by the other metal ions tested, Co(II), Ni(II), Cd(II) and Ca(II), notwithstanding the interaction with *N*-carboxymethyl chitosan revealed by the thermodynamic techniques for some of them. In this respect, Cu(II) and Pb(II) qualify as very interesting specific probes in studies of the binding of metal ions by polycarboxylate systems, as already emphasized by Cesàro *et al.* (1988*a*,*b*)

The values of molar UV absorbance and ellipticity at wavelengths corresponding to the maximum of the related bands are reported for these systems as a function of *R* in Figs 7 and 8, respectively, where the differences between copper and lead appear even more evident, especially in view of the saturation point for Cu(II). As already done with the calorimetric curves, the binding ability can be evaluated again by means of the Scatchard method. The whole set of binding constant values so derived is reported in Table 1.

CONCLUSIONS

The values presented in this paper, and hence the trend of the affinity of the metal ions towards *N*-carboxymethyl chitosan, have been obtained in dilute solutions, where the binding parameters are mainly influenced by the chemical nature of the reacting species In contrast, when working with highly concentrated or cross-linked systems a trend in the binding affinity is more difficult to detect It is the case, for instance, with the chelation isotherms reported by Muzzarelli *et al* (1985) and by Muzzarelli & Zattoni (1986) for insoluble substituted chitosans, includ-

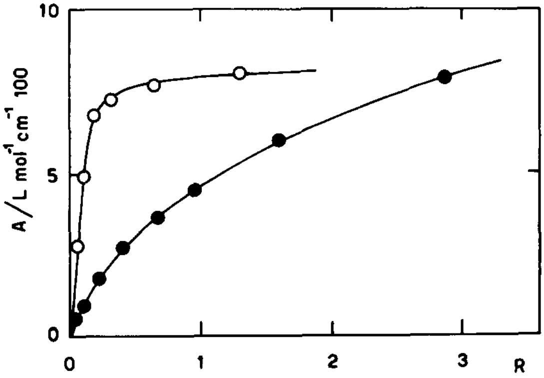

Fig. 7. Molar absorbance change as a function of metal cation-to-polymer molar concentration, *R* ○, Cu(II), $\lambda = 246$ nm, ●, Pb(II), $\lambda = 234$ nm

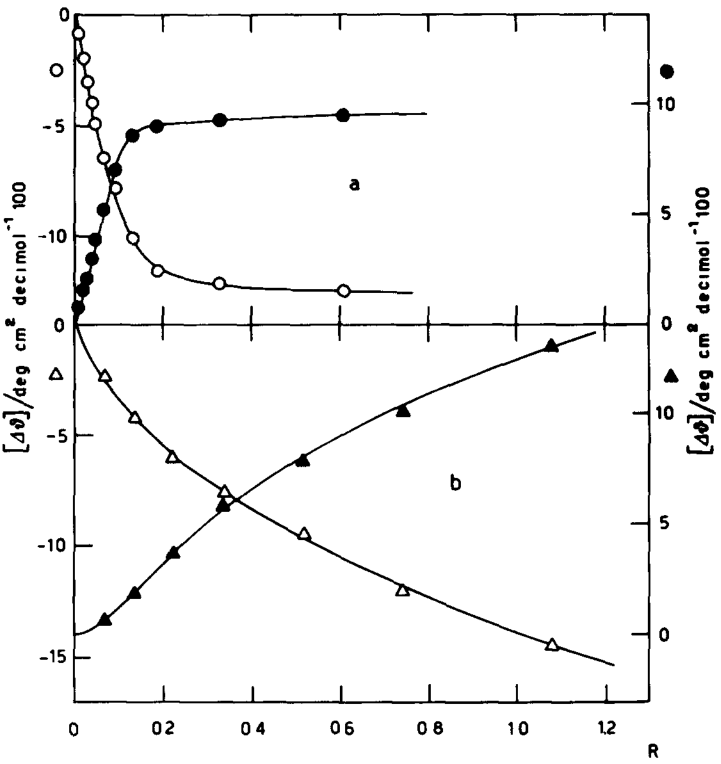

Fig. 8. Molar ellipticity change as a function of metal cation-to-polymer molar concentration, *R* (a) Cu(II) ○, $\lambda = 277$ nm, ●, $\lambda = 237$ nm (b) Pb(II) △, $\lambda = 244$ nm, ▲, $\lambda = 231$ nm

ing insoluble forms of *N*-carboxymethyl chitosan, in those cases, cross-linking brings about an enhancement of the binding ability, with an evident levelling effect likely due to the high local concentration of the chelating groups. In any case, good agreement exists between the data presented here and those given in terms of adsorption capacity of insoluble forms of *N*-carboxymethyl chitosan (Muzzarelli *et al*, 1985, 1989)

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