

N-(*o*-CARBOXYBENZYL) CHITOSAN, *N*-CARBOXYMETHYL CHITOSAN
AND DITHIOCARBAMATE CHITOSAN:
NEW CHELATING DERIVATIVES OF CHITOSAN

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Abstract - Dithiocarbamate chitosan, an insoluble derivative obtained from carbon disulfide, and *N*-(*o*-carboxybenzyl) chitosan and *N*-carboxymethyl chitosan, two water-soluble polyampholytes obtained from phthalaldehydic acid and glyoxylic acid, can be used for removal of metal ions from dilute solutions. Their characteristic properties are described in terms of infrared, ultraviolet and circular dichroism spectrometry, alkalimetry, metal ion collection percentage and metal ion capacity. The new polyampholytes form insoluble chelates upon addition of their solutions to metal ion solutions.

INTRODUCTION

The chelating ability of chitosan for transition metal ions (Ref. 1-3) can be enhanced by reacting the amino groups of this biopolymer with suitable chemicals (Ref. 4). Various chemical derivatives of chitosan have thus far been prepared: in this lecture, we shall devote our attention to three derivatives recently prepared in our laboratory (Ref. 5-8) which appear to represent significant advances in the field of the selective sorption of metal ions from dilute solutions and a new methodological approach to the problem of metal ion recovery. These three new derivatives are: dithiocarbamate chitosan (DTCC), *N*-(*o*-carboxybenzyl) chitosan (NCBC) and *N*-carboxymethyl chitosan (NMC), the first of which is a water-insoluble powder that lends itself to be used in batch and in column operations; the latter two are water-soluble polyampholytes which yield insoluble chelates of many metal ions.

While the collecting ability of DTCC is mainly due to its high sulfur content which leads to the formation of very stable sulfur compounds of the metal ions, the collecting ability of NCBC and NMC is due to the combined actions of acetamido, secondary amino and carboxyl groups in the chelating reaction.

Similar derivatives of other polymers carrying amino groups have also been studied by other research teams: thiol and dithiocarbamate functionalities were introduced into polymers prepared by polycondensation of polyethylenimine and cyanuric chloride in the presence of thiourea (Ref. 9) and into various aminocelluloses treated with carbon disulfide, respectively (Ref. 10).

Chelating polyampholytes, however, such as NCBC and NMC, are quite new products in so far as they are very soluble, due to their carbohydrate backbone and oppositely charged groups, and as they interact with the metal ions in homogeneous phase, thus favoring the complexation of the metal ions.

We shall examine several characteristic properties of DTCC, NCBC and NMC and discuss their ability of removing metal ions from dilute solutions.

DITHIOCARBAMATE CHITOSAN

Chitosan readily reacts with carbon disulfide to produce derivatives possessing distinct characteristics. Modifications of the free amino groups of chitosan to give -NH-CS-S^- groups alter the elemental composition of the polymer and the macromolecular conformation as well, mainly because of hydrogen-bond rupture.

The infrared spectra of DTCC exhibit bands at 1480 (assigned to N-C=S) and 940 cm^{-1} , with evidence of generalized alterations in the $1400\text{--}1550\text{ cm}^{-1}$ interval. The ratios of the absorbances at 1650 cm^{-1} and 1370 cm^{-1} (not altered with respect to untreated chitosan), and those denoting alterations at 1480 and 940 cm^{-1} , follow the trend in Fig. 1. It may be noted that these ratios drastically decrease during the initial 5 hours of reaction, and thus it seems that DTCC formation is rather rapid.

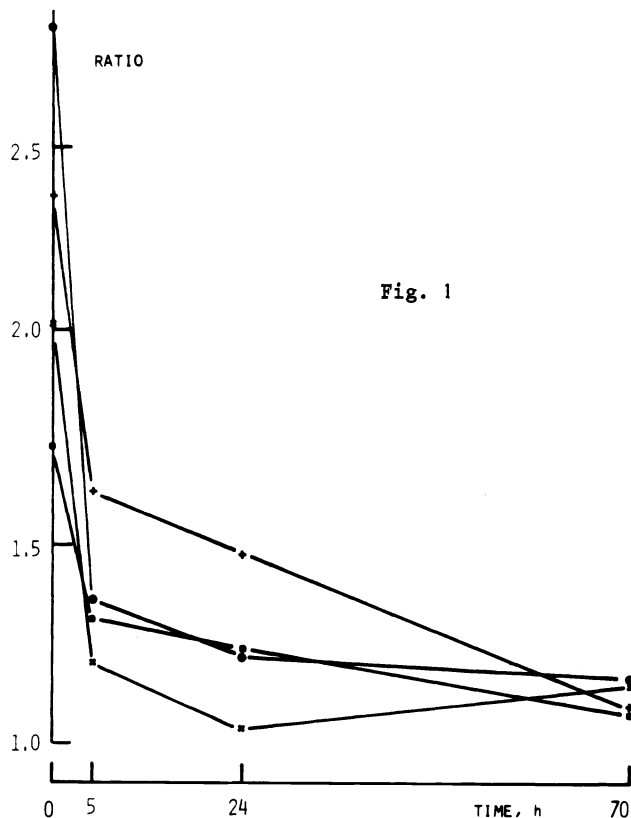


Fig. 1

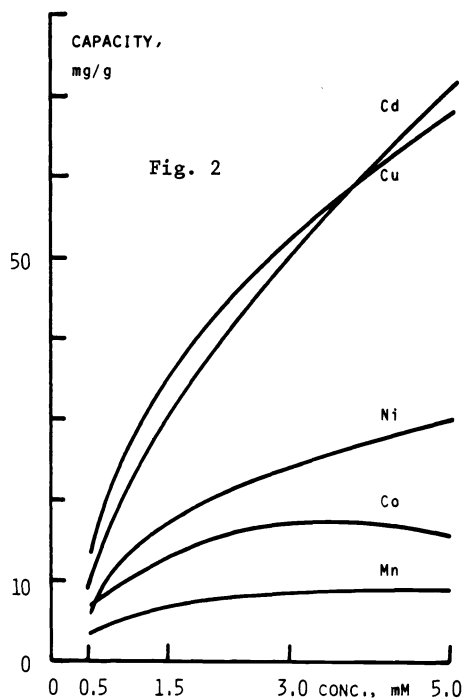


Fig. 2

Figure 1. Ratios between the infrared bands at (●) 1650/1480; (×) 1370/1480; (+) 1650/940, and (■) 1370/940 cm^{-1} , showing that the formation of DTCC occurs in about 5 h.

Figure 2. Capacity of DTCC for metal ions at 20°C and pH 6.8, as a function of the metal-ion concentration (100 mg DTCC/25 ml).

The x-ray diffraction spectra reveal significant alterations depending on the time of contact of chitosan with the reaction mixture. The 2θ readings for the krill chitosan, progressively changed from $9^\circ13'$ and $19^\circ58'$ (0.958 and 0.444 nm) to $8^\circ13'$ and $19^\circ58'$ (1.074 and 0.444 nm) after 70 h. While the e.s.r. spectrum of the original krill chitosan is a featureless single line, those recorded for DTCC show a sextet of signals whose coupling constant is 85 G. Moreover, five couples of signals are present having constants 25–30 G. The experiments described here were conducted mostly on DTCC containing 7.8 % sulfur.

Allowing for a moisture content of 5 %, the degree of substitution for a polymer containing 7 % sulfur was: 21 % dithiocarbamate, 39 % free amine, and 40 % acetamido; for a polymer containing 11 % sulfur was: 35 % dithiocarbamate, 25 % free amine, and 40 % acetamido.

Transition metal ions are efficiently collected by DTCC. Numerous measurements by atomic absorption spectrometry showed that both cations and amino complexes in the pH interval tested (5.0–12.0) are totally retained by DTCC. At pH 3.0, retention is rather low but still appreciable for most of the cations studied. However, tests on cobalt and manganese performed with DTCC powder kept on filter paper for 3–11 days, indicate that the percentage of collection decreases, presumably because of oxidation

and hydrolysis: DTCC should therefore be stored in sealed bottles. The exceptional collection ability of DTCC is evident in the case of amino complexes of copper, nickel, cobalt and cadmium, which are totally collected within one hour of contact and impart typical colors to the white powder, as described in Table I.

The capacity of DTCC was studied as a function of the concentration of metal ion in the range 0.5–10.0 mmol/l, at three pH values: 3.0, 6.8 and 12.0 (final values). Even at pH 3.0, the capacity of DTCC is appreciable for a number of cations, especially cobalt-II, nickel-II, copper-II and cadmium-II (more than 5 % w/w for the latter two). At pH 6.8, the capacities indicated in Fig. 2 at 5 mmol/l metal-ion concentration are 0.8 % for Mn, 1.5 % for Co, 3.0 % for Ni, 4.0 % for Cr, 7.2 % for Cd and 6.7 % for Cu. In ammonia solutions (pH 12.0), the respective capacities are 3.7 % for Co, 3.8 % for Ni, 4.7 % for Cu and 11.7 % for Cd. Within the experimental errors, the DTCC's obtained after various times of reaction possess the same capacities.

Table I

Colors and collection percentages of the DTCC chelates obtained from 0.5 mmol/l solutions (100 mg of 100–200 mesh powder stirred with 25 ml solution for 1 h), at various pH values adjusted with sulfuric acid or ammonia. Colors appear immediately after contact.

Metal ion	pH 1		pH 3		pH 5–7		pH 10.5–12	
	Color	Collection %	Color	Collection %	Color	Collection %	Color	Collection %
Chromium-III	pale yellow	0	grey	4	pale green	96		
Manganese-II	pale yellow	25	light brown	13	light brown	93		
Cobalt-II	green	14	dark green	71	dark green	95	brown	100
Nickel-II	yellow		green	77	green	100	green	100
Copper-II	brown	90	brown	100	brown	90	dark green	100
Zinc-II	white		white		white	100	white	
Cadmium-II	white	10	yellow	100	yellow	100	yellow	100
Lead-II					brown	100		
Silver-I					black	100		
Mercury-II					light brown	100		
Uranyl					orange	100		

It was therefore anticipated that chromatographic columns would effectively remove metal ions from waters. Fig. 3 shows the saturation curve obtained with 10 $\mu\text{g/g}$ Cu^{2+} solution at pH 9.5 (initial value). At this level, copper is totally removed from a mass of water 100 times larger than the DTCC in the column; the resulting breakthrough curve is not very steep.

Similarities exist between DTCC and dithiocarbamate cellulose: for instance, the capacities for copper are 8 mg Cu/g DTCC (0.5 mmol/l) and 4 mg Cu/g DTC-cellulose (0.125 mmol/l); those for mercury are 25 mg Hg/g DTCC (0.5 mmol/l) and 20 mg Hg/g DTC-cellulose (0.1 mmol/l) under comparable conditions (Ref. 10). A similarity also exists in the high 100 % or close) collection percentages for the ammonia complexes at pH 10.0–10.5 (compare Table I of this work with Fig. 1 of Ref. 10). A striking difference is the collection percentage of cobalt: at pH 3 it is 71 for DTCC, but only 5 for DTC-cellulose; for cadmium at pH 3 is 100 for DTCC against 65 for DTC-cellulose.

Where the experimental conditions adopted permit, a comparison may also be made with the polymers obtained from cyanuric chloride treated with polyamines and thio-

urea: at 0.5 mmol/l concentration, copper is completely retained, even at pH 3 (compare Table II and Fig. 3 of Ref. 5 with Figs. 1 and 2 of Ref. 9), however, the capacity of DTCC is much higher (50 mg Cu/g DTCC at 5 mmol/l Cu) when compared with 32 mg Cu/g DTC-cellulose at 16 mmol/l Cu.

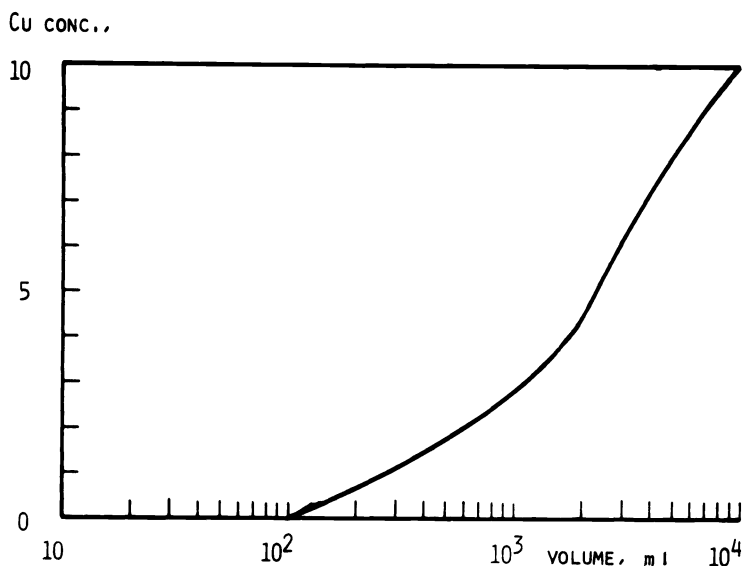


Figure 3. Breakthrough curve for a 10 µg/g copper-11 neutral solution fed to a 0.6 × 10 cm column at the flow-rate of 0.5 ml/min.

The DTCC is much easier to prepare than DTC-cellulose because the latter requires preparation of amino cellulose from O-tosyl cellulose; the same may be said for polymers prepared from cyanuric chloride in hazardous solvents.

DTCC is thus superior to chitosan and to the derivatives so far described, including thiourea and S-acetylmercaptosuccinic anhydride derivatives, as far as manganese, cobalt and nickel at pH 2.5 and 5.5 are concerned, because their collection values are generally higher for DTCC than for the derivatives indicated and for chitosan itself (Compare Table I in this work with Table I of Ref. 4).

N-(*o*-CARBOXYBENZYL) CHITOSAN AND *N*-CARBOXYMETHYL CHITOSAN

Our original approach to the carboxymethylation of chitosan (Ref. 7) consists in reacting the free amino groups of chitosan with glyoxylic acid, to produce a soluble gel-forming imine and then reducing it with any proper reducing agent, such as NaBH₃CN. This preparation is very easy to carry out, does not require cooling or warming, and only requires commercially available reagents. The resulting products are a class of NCMC's, containing acetylated, carboxymethylated and free amino groups in proportions easily controlled through the choice of the starting chitosan (degree of acetylation and molecular weight) and the amount of glyoxylic acid used. Phthalaldehydic acid is also suitable for the preparation of chitosan-derived polyampholytes because the condensation reaction is instantaneous and the hydrogenation of the aldimine is very easily performed (Ref. 6 and 8).

Chitosans modified via reactions at the free amino groups have been described in a number of papers and include derivatives of glutaraldehyde (Ref. 4), *N*-acylchitosans (Ref. 11 and 12), *N*-alkylidene chitosans (Ref. 13) and branched-chain chitosans (Ref. 14); however, we were the first to report on chitosan derivatives possessing the characteristic properties of chelating polyampholytes (Ref. 7).

The infrared spectra of *N*-(*o*-carboxybenzyl) chitosan recorded upon insolubilization of the polymer at various pH values (1.2, 2.4, 4.0, 8.0 and 11.8) exhibit the 750 cm⁻¹ band assigned to the aromatic C-H. Absorption bands due to the primary and secondary amines and to the amide are also present at 1600, 1580 and 1550 cm⁻¹. Aro-

matic o-substituted COOH presents absorption bands around 1690 cm^{-1} . Absorption bands due to the amido group are also present in this region and therefore the aromatic COOH band is not sharply distinguishable. In going from acidic to alkaline pH values a remarkable absorption increase is observed at 1580 and 1410 cm^{-1} , due to the carboxylate anion. A concurrent decrease of the absorption band at 1690 is also observed because of the ionization of the carboxyl group. The infrared spectra, therefore, confirm the presence of aromatic rings and carboxyl groups in the NCBC preparations.

The infrared spectra of fully substituted NCMC samples were recorded at various pH values: 2.2, 2.7, 6.5 and 12.0. The 1730 cm^{-1} band due to COOH appears at pH 2.7, and it is clearly present at pH 2.2, while at higher pH values it is absent. In going from pH 2.2 to pH 12.0, the band at 1730 cm^{-1} decreases while those at 1580 and 1400 due to CO_2 increase as a consequence of the ionization of $-\text{COOH}$ to $-\text{COO}^-$, particularly above pH 2.7 where the polymer is in the form $\text{Chit}-\overset{+}{\text{N}}\text{H}_2-\text{CH}_2-\text{COO}^-$.

Phthalaldehydic acid solutions brought to pH 1.6 with hydrochloric acid present absorption maxima at 203.5, 231.5, 273.0 and 280.0 nm. The same solutions brought to pH 12.5 with NaOH present absorption maxima at 216.0, 252.0 and 292.0 nm. NCBC solutions brought to pH 1.6 with HCl present absorption maxima at 204.0, 230.0 and 274.0 nm, while in alkaline solutions absorption occurs at 220 nm only. The absorption band at 274.0 nm is therefore suitable for the determination of the substitution degree of NCBC as well as for the analytical determination of NCBC in solutions.

Since the chitosan used was known to have the degree of deacetylation of $58\pm 4\%$, according to infrared spectrometry, the NCBC, if fully carboxybenzylated, would have the same degree of carboxybenzylation ($58\pm 4\%$) and the same degree of secondary amine. A phthalaldehydic acid solution prepared in such a way as to have the same concentration as a 0.2 % solution of NCBC in terms of aromatic units with the assumption of a 58 % degree of carboxybenzylation was used to draw a calibration curve against which the 0.2 % solution of NCBC was read: the degree of carboxybenzylation referred to the free amine available was then found to be 74 %, which means $43\pm 3\%$ of the total. The NCBC concentration was also read at 274.0 nm in order to determine the concentration of NCBC left in solution after metal ion chelation. At this wavelength, the metal ions do not absorb, thus these determinations were performed in conjunction with the metal ion collection measurements.

The alkalimetric curves of NCBC contained inflection points, the first of which (pH 3.9) was due to the complete titration of carboxyl groups: at this point, the carboxyl groups were completely dissociated, while the amines were protonated. The second inflection point (pH 6.8) was attributable to the neutralization of the primary amine or part of the secondary amine, in such a way that equal numbers of negative and positive charges were present on the polysaccharide chain. It corresponded to the isoelectric point, and, in fact, it was in the middle of the precipitation interval. The third inflection point (pH 9.5) was attributable to the complete neutralization of the amine functions.

The first and second inflection points were 4.0 ml apart; the second and third, 6.6 ml apart. 10.6 ml of 0.1 mol/l NaOH were therefore necessary for the titration of the whole amine functionality. All these data correspond to NCBC possessing the following characteristics: degree of deacetylation $42\pm 4\%$, carboxybenzylation $36\pm 4\%$; free amino groups $22\pm 2\%$. Water, $16\pm 1\%$ by weight. The pH values were 5.7 and 8.0.

On the titration curve obtained with fully substituted NCMC's the inflection points were 2.0, 4.1 and 9.5; the first one was due to the titration of HCl in the presence of a fully protonated polymer; the second corresponded to the titration of the carboxyl group of the polymer, whose form at pH 4.1 (isoelectric point) is $-\overset{+}{\text{N}}\text{H}_2-\text{CH}_2-\text{COO}^-$, as confirmed by infrared spectrometry; the third one corresponded to the complete titration to $-\text{NH}-\text{CH}_2-\text{COO}^-$. The samples treated with excess glyoxylic acid were found to be fully carboxymethylated, to the extent allowed by their degrees of deacetylation.

Evidence of the chelation reaction of NCBC and NCMC was obtained by circular dichroism spectropolarimetry. The spectra of the polysaccharides at various pH values are those in Figures 4 and 5; while NCMC shows a negative Cotton band around 210 nm, which is typical of the acetamido groups (Ref. 15), the NCBC spectrum does not possess such a band, but shows two negative Cotton bands, around 230-240 nm and

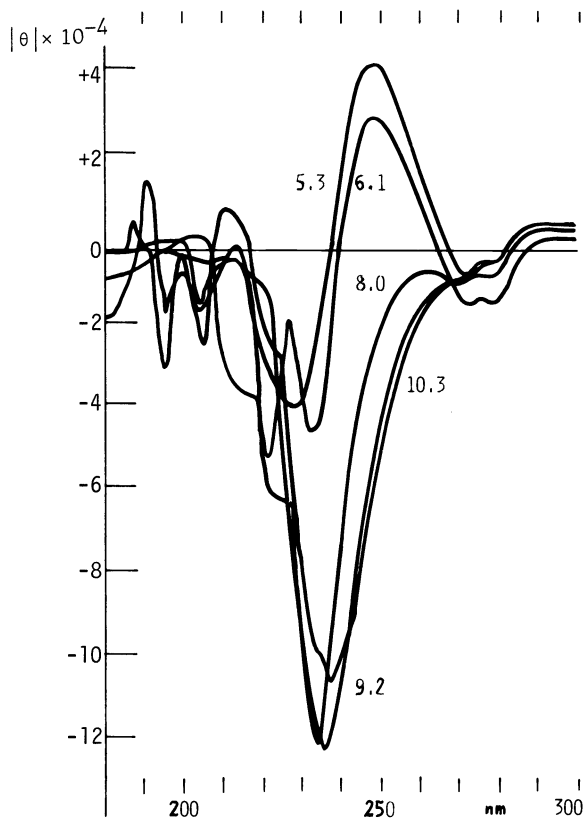


Figure 4. Circular dichroism spectra of 0.05 % solutions of NCBC at pH values 5.3, 6.1, 8.0, 9.2 and 10.3 (prepared by diluting a 0.5 % NCBC solution after addition of the proper amounts of acetic acid and sodium hydroxide). Degrees centimeter² decimole⁻¹ versus nanometers.

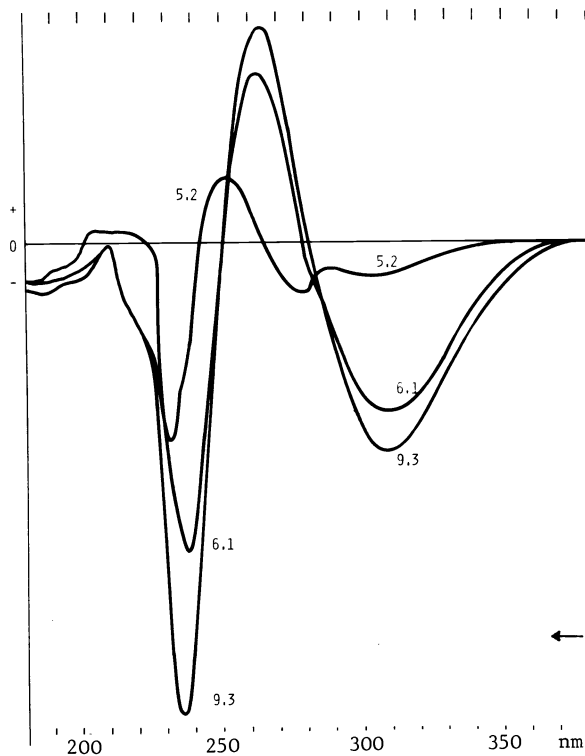


Figure 6. Circular dichroism spectra of 0.05 % solutions of NCBC at pH values 5.2, 6.1 and 9.3, in the presence of 0.2 mM copper sulfate. Slightly turbid solutions.

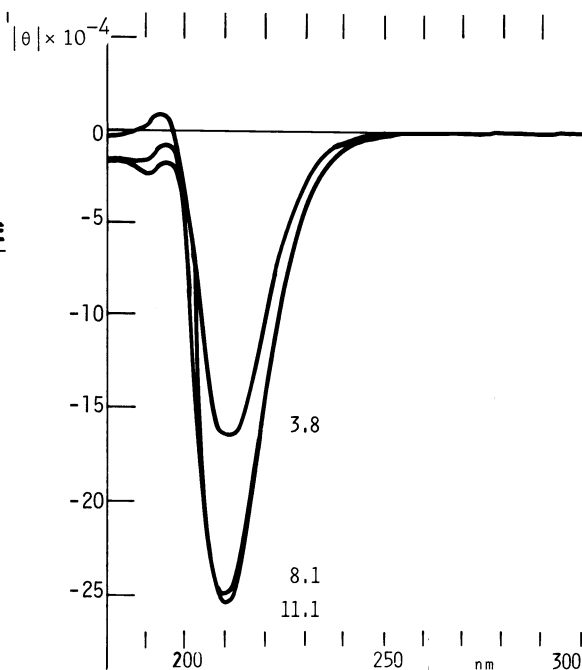


Figure 5. Circular dichroism spectra of 0.05 % solutions of NCMC at pH values 3.8, 8.1 and 11.1 (prepared by diluting a 0.5 % NCMC solution, after addition of proper amounts of acetic acid and sodium hydroxide). Degrees centimeter² decimole⁻¹ versus nanometers.

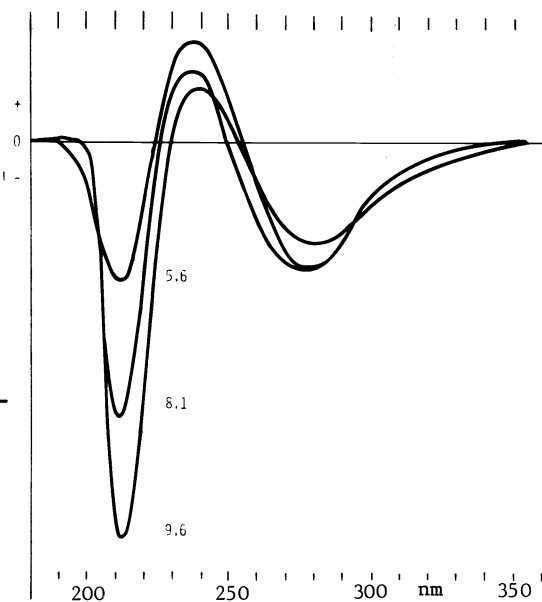


Figure 7. Circular dichroism spectra of 0.05 % solutions of NCMC at pH values 5.6, 8.1 and 9.6, in the presence of 0.2 mM copper sulfate. Turbid solutions.

270–280 nm, together with a positive band at 250 nm, induced by the polysaccharide backbone on the aromatic group.

Upon reaction with cupric ions, the NCBC samples at all pH values (5.2, 6.1 and 9.3) exhibit the positive band progressively shifted to 264 nm accompanied by negative bands at 300–310 nm, due to the chelate (Figure 6). The chelation of cupric ions by NCMC leads to analogous alterations: a novel positive band at 235–240 nm and a novel negative band at 270–280 nm appear in the circular dichroism spectra: they are sensitive to the pH values as shown in Fig. 7.

One of the characteristic properties of NCBC and NCMC is, in fact, the ability to chelate transition metal ions, thus yielding insoluble chelates, which readily settle as hydrated solids within minutes after mixing. Even very dilute solutions (0.1 mmol/l) can be conveniently treated with these soluble polyampholytes to collect transition metal ions: the supernatant can be removed by paper filtration or centrifugation.

The insolubilization of metal ions by chelation with soluble NCBC is pH-dependent. In the cases of Cr, Cu, Zn, Hg and Pb, maxima are observed at neutrality, while Co and Ni exhibit maxima at pH 8.5. In all cases, with the exception of Cd and U, a sharp decrease of collection percentage is observed at alkaline pH values. This fact indicates that the highest yields can be reached by proper pH adjustment.

For instance, in Fig. 8, the pH dependence of the collection percentage is reported for copper. The collection percentages plotted in Fig. 9 have therefore been measured

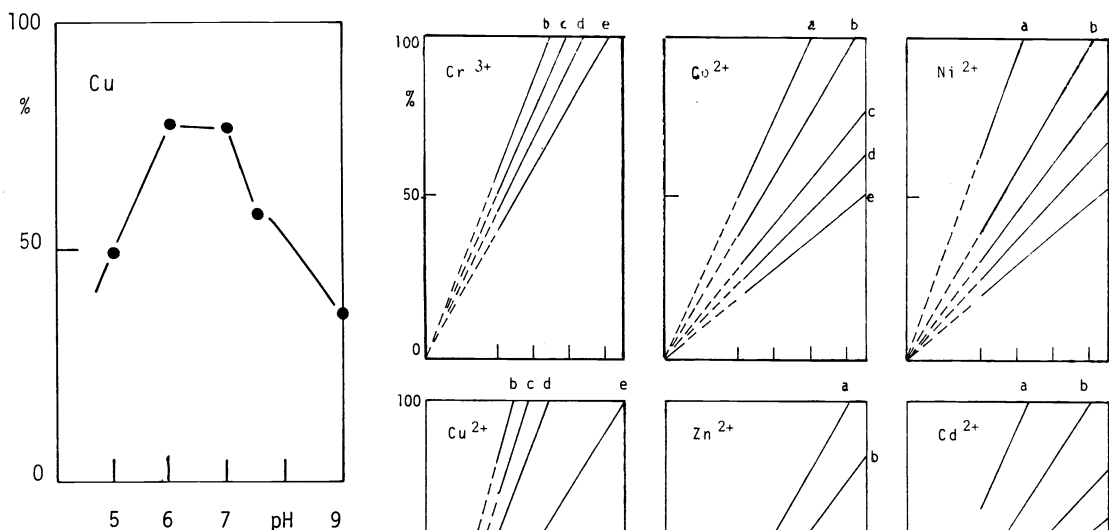
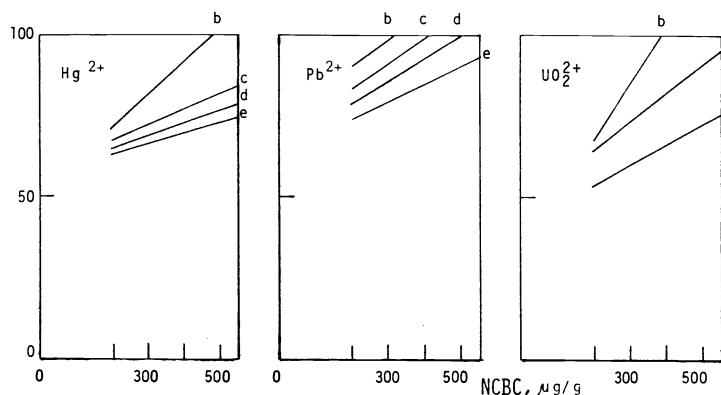


Figure 8. Collection percentage of cupric ions on NCBC vs pH value.

Figure 9. Collection percentages of various metal ions in aqueous solutions (a = 0.1, b = 0.2, c = 0.3, d = 0.4 and e = 0.5 mmol/l) on NCBC vs NCBC concentration ($\mu\text{g/g}$).



on solutions whose pH was within a restricted interval of values. A linear dependence of the collection percentages on the polymer concentration was observed in almost all cases even when low metal ion concentrations (0.1 mmol/l) were studied. NCBC concentration (around 200–300 $\mu\text{g/g}$ could scavenge Cr, Ni, Cu, Cd, Pb and Hg from their respective 0.1 mmol/l solutions.

In the NCBC concentration interval studied, Cr, Co, Ni, Cu, Cd, Pb and U could be scavenged from 0.1 and 0.2 mmol/l solutions. Cr, Cu and Pb could also be completely removed even when present at higher concentrations (0.3, 0.4 and 0.5 mmol/l).

The concentration of NCBC left in aqueous phase after chelation and filtration was measured by u.v.-vis. spectrophotometry. After the complete removal of the metal ions, under the conditions adopted, some NCBC remains, for instance after the complete removal of Co, with 400 $\mu\text{g/g}$ NCBC, 50 $\mu\text{g/g}$ of NCBC are found in solution. After the complete removal of Ni and Cd, with 300 $\mu\text{g/g}$ NCBC, 25 $\mu\text{g/g}$ and 10 $\mu\text{g/g}$ of NCBC remain in solution, respectively. For instance, small amounts of NCBC (200 mg) used on 1 l of solution lowered the Cd concentration from 0.100 mmol/l to 0.033 mmol/l; when 300 mg of NCBC were used, the Cd concentration was lowered to 0.010 mmol/l and the NCBC left was 13 $\mu\text{g/g}$.

Since the molecular weight of the average repeating unit of the NCBC sample here considered is 236, one mole of carboxybenzylamine residue is associated with 567 dalton

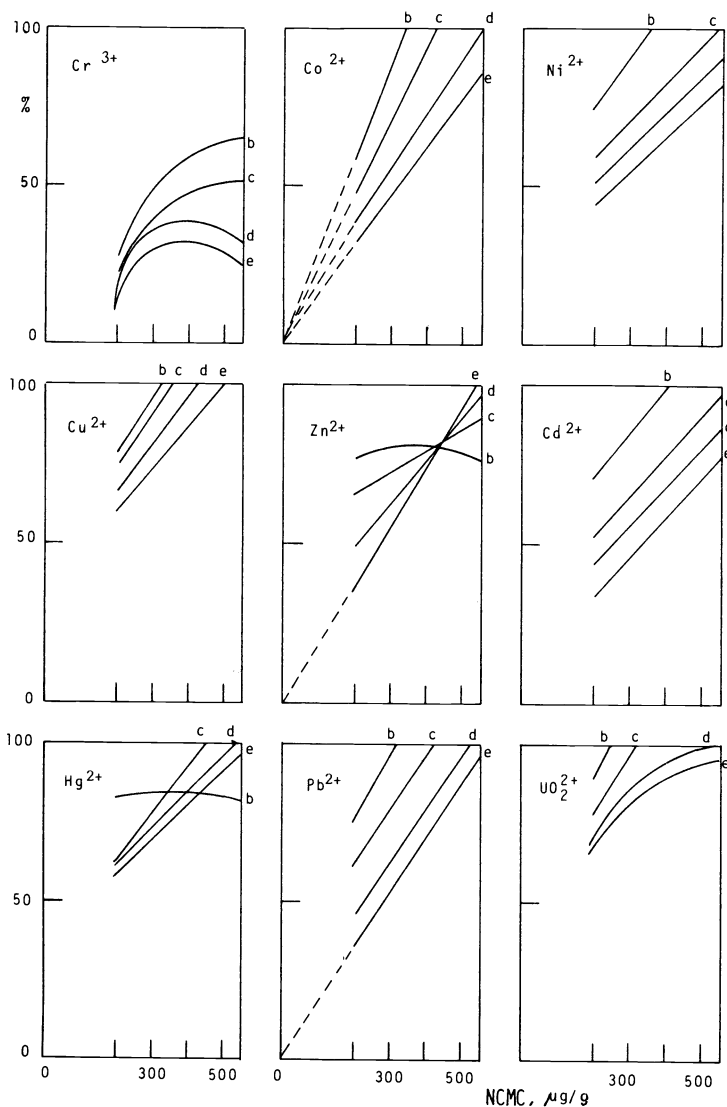


Figure 10. Collection percentages of various metal ions in aqueous solutions (a = 0.1, b = 0.2, c = 0.3, d = 0.4 and e = 0.5 mmol/l) on NCBC vs NCBC concentration ($\mu\text{g/g}$).

(based on the above data and assuming the value of 40% as the most probable carboxybenzylation degree), the molar ratios between carboxybenzyl units and metal ions can be calculated from the data in Fig. 9. Of course, they depend on concentrations: however, they are found to be close to 3 for Co, Ni and Cd; around 2 for Pb and Hg and about 1.5 for Cu.

The insolubilization of metal ions by chelation with soluble NCMC is also pH-dependent. In the cases of Co, Cu, Zn, Hg and Pb, maximum insolubilization is observed at neutrality (pH 6-7), while Ni and Cd have maxima at pH 7.5 and U at 5.5. The collection percentages, plotted in Fig. 10, have been therefore measured on solutions whose pH was within a restricted interval of values. A linear dependence of the collection percentages on the NCMC concentration was observed in almost all cases, even with low metal ion concentrations (0.2 mmol/l). NCMC in the concentration interval studied completely scavenged Co, Ni, Cd, Pb and U from 0.2 mmol/l solutions; Cu, Hg, Pb and U could also be completely removed even when present at higher concentrations (0.3-0.5 mmol/l).

Since the average molecular weight of the repeating unit of the fully substituted NCMC is 212, one mole of carboxymethylamine residue is associated with 378 dalton (based on the data above and assuming the degree of carboxymethylation 56 %); thus, the molar ratios between carboxymethylamine residues and metal ions are close to 3. Of course, they depend on concentrations and on contributions by other groups to the chelation.

COMPARING SOLUBLE AND INSOLUBLE DERIVATIVES OF CHITOSAN

According to the above presentation, chemical derivatives of chitosan can be distinguished as soluble chelating polyampholytes and insoluble chelating powders. Many points of comparison have already been mentioned, however, let's now stress their

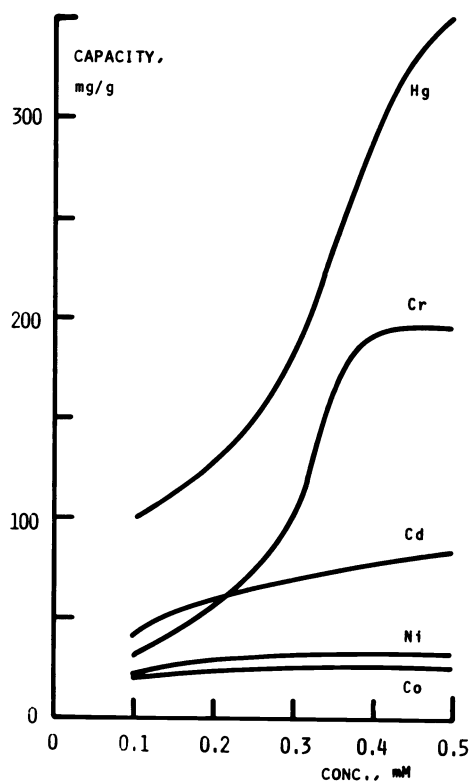


Figure 11. Capacities of NCMC for metal ions at 20°C and pH 6.8, as a function of the metal ion concentration (as obtained after precipitation of insoluble chelates). Compare with data in Fig. 2; note different values on the abscissa.

differences in terms of metal ion capacity. Since NCBC and NCMC are applied to the metal ion solutions as solutes, whilst DTCC is contacted with metal ion solutions as a powder, according to classical techniques (batch or column), it may be expected that the soluble polyampholytes would yield a final chelate whose capacity (mg metal/g polymer) is higher than for the insoluble powders. Within one hour after contact, the removal (collection percentage) is higher with both kinds of polymers, and, in fact, with dilute solutions, the capacity of NCBC and NCMC are strikingly higher than for DTCC.

If we refer to a 0.5 mmol/l solution of metal ion, the capacity of DTCC at pH 6.8 are less than 10 mg/g for Ni, Co and Cd (see Fig. 2) while those for NCBC are 22, 26 and 80, respectively (Fig. 11). With NCBC and 0.5 mmol/l solutions, capacities as high as 200 mg Cr/g, 350 mg Hg/g and 781 mg Pb/g can be easily reached.

The removal of metal ions from dilute solutions with the aid of soluble chitosan-derived polyampholytes may become an attractive technique, because of its simplicity and minimum amount of polymer used, while derivatives such as DTCC may be of help when dealing with complexed metal ions.

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