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N-(*o*-CARBOXYBENZYL) CHITOSANS: NOVEL CHELATING POLYAMPHOLYTES

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ABSTRACT

The imine formed by chitosan with phthalaldehydic acid was reduced with sodium cyanoborohydride and the resulting N-(o-carboxybenzyl) chitosan (NCBC) was insolubilised with ethanol and acetone and obtained as a white, free-flowing powder, soluble in both acidic and alkaline solutions. A sample of NCBC with the following degrees of substitution: acetamido 42% ± 4%, N-(o-carboxybenzyl) amine 43% ± 3%, free amine 15% ± 1% and containing 16% ± 1% moisture, was characterised by IR and UV–Vis. spectrometry, titration and viscometry. The isoelectric point was 6·8; the pK_a *values were 5·7 and 8·0. NCBC could be determined by UV–Vis. spectrophotometry in aqueous solutions at 274 nm; maximum viscosity of the solutions was observed at pH 4. Upon addition of NCBC to transition metal ion solutions (0·1–0·5 mM) chelation and insolubilisation took place immediately. The dependence of the collection percentage on pH, NCBC and metal ion concentrations was studied for nine metal ions.*

INTRODUCTION

Plisko *et al.* (1972) reacted chitosan with salicylaldehyde to protect the amino groups of chitosan as a preliminary step to the carboxymethylation of alkali-chitosan with sodium monochloroacetate (Muzzarelli, 1977). The rationale was to offer an alternative to the production of *o*-carboxymethyl chitosan (OCMC) from alkali chitin since deacetylation and depolymerisation then occurs during the course of reaction with sodium monochloroacetate in concentrated alkali yielding non-reproducible products.

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In spite of these difficulties Hall & Yalpani (1980*a*) and Koshugi (1980*a,b,c*; 1981*a,b,c*) have produced and studied OCMCs from alkali chitin. Hirano & Moriyasu (1981) and Mrachkovskaya *et al.* (1981), following a different approach, obtained *N*-carboxyacyl chitosans by reaction with dicarboxylic acid anhydrides.

Muzzarelli (1981; 1982, 1983) and Muzzarelli *et al.* (1981*a,b*; 1982) have shown that a straightforward way to prepare carboxymethyl chitosan is by the formation of an aldimine with glyoxylic acid, CHO.COOH, and then reduction with cyanoborohydride. The resulting product is *N*-carboxymethyl chitosan (NCMC), i.e. a polyampholyte with varying degrees of primary amine, secondary amine, acetamido and carboxyl functions, depending on the preparative conditions adopted. Chitosan aldimines have been prepared by Moore & Roberts (1981).

While it is apparent that other polyampholytes can be prepared from chitosan, no information has so far been published other than that relevant to OCMC and NCMC.

The interesting chelating properties of these polyampholytes has been pointed out by Muzzarelli (1977, p. 122). Limited data on copper chelation was presented recently by Hall & Yalpani (1980*b*) and by Takahashi *et al.* (1978). The metal complexes of aldimines obtained from substituted salicylaldehydes and glucosamine, galactosamine and mannosamine were studied by Maeda & Tsuji (1979).

In view of the desirable properties possessed by soluble polyampholytes derived from carbohydrates, we have undertaken the study of the reaction of chitosan with phthalaldehydic acid, $C_6H_4CHO.(o)COOH$. This compound 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. This paper reports the original preparation of two new classes of chitosan derivatives, namely *N*-(*o*-carboxybenzylidene) chitosans and *N*-(*o*-carboxybenzyl) chitosans, and the characterisation of the latter with particular reference to chelating behaviour.

EXPERIMENTAL

Reagents. Chitosan from *Euphausia superba* (Antarctic krill), deacetylation degree 58 ± 4% (Muzzarelli *et al.*, 1981*a*) was supplied by Rybex, Sczesczyn, Poland. Phthalaldehydic acid and sodium cyanoborohydride were supplied by Merck-Schuchardt.

Viscometry. Viscosities were measured with a Haake Rotovisco RV 12 viscometer, equipped with a programmer, a Hewlett-Packard recorder and the Haake thermostat-cryostat; various concentric cylinder geometries were used and the data interpreted as described by Muzzarelli *et al.*, 1981*a*.

Infrared spectrometry. Spectra were recorded with a Perkin-Elmer infrared spectrometer Model 299-B, on translucent discs obtained by pressing the ground material in admixture with KBr using the Perkin-Elmer press. Five 0·2% (w/v) NCBC solutions were adjusted to pH 1·2, 2·4, 4·0, 8·0 and 11·8; the polysaccharide was then precipitated with acetone, and washed with ethanol and acetone.

Atomic absorption spectrometry. Analyses were carried out with the Perkin-Elmer 305 spectrometer, equipped with flame and graphite atomisers, according to standard methods.

Metal ion chelation and insolubilisation. Solutions (0·1, 0·2, 0·3, 0·4 and 0·5 mM) of the sulphates of the divalent cations of cobalt, copper, zinc, manganese and caesium; uranyl sulphate, chromium-III sulphate, mercury-II chloride and lead-II nitrate were prepared. Four 50 ml aliquots of each solution containing, respectively, 10, 15, 20 and 25 mg of NCBC were agitated on a vortex machine at 80 rpm for 1 h, then filtered through Whatman filter paper and the filtrate analysed by atomic absorption spectrometry.

Titration. An AMEL pH meter was used in conjunction with Titrisol 0·1 M HCl and NaOH solutions. The NCBC samples (0·5 g) were dissolved in 0·1 M HCl (50 ml) and titrated with 0·1 M NaOH under nitrogen. Sufficient time was allowed between additions to permit accurate readings, especially in the central part of the pH range.

UV–Vis. spectrophotometry. A Perkin-Elmer spectrophotometer Model 544 was used with the following settings: slit 2 nm; ordinate ABS; scale 0–3; speed 120 nm/min; chart 20 nm/cm.

Preparation of NCBC. Chitosan phthalaldehyde solutions were obtained by adding the desired amount of phthalaldehydic acid (11·20 g in 1 litre water) to a stirred suspension of chitosan powder (20·00 g in 1 litre water). Stirring was continued for ½ h and concentrated HCl (10 ml) added to dissolve the chitosan. Any insoluble particles present were removed by filtration using a Buchner funnel. The pH value was adjusted to 4·1 with NaOH to permit formation of the Schiff base. The resulting solution contained soluble *N*-(*o*-carboxybenzylidene) chitosan. The reducing agent, sodium cyanoborohydride (4·24 g in about 50 ml water) was then added with stirring and the pH rose to 7·5. Finally, the pH was brought to 8·0 with NaOH. The resulting *N*-(*o*-carboxybenzyl) chitosan gel was left standing overnight and then adjusted to pH 6·8 (the isoelectric point) with HCl. The water soluble polyampholyte was precipitated by the addition of ethanol (1·5 litre) and acetone (2·0 litre). The final product, a white powder, was purified by extracting in Soxhlet extraction with ethanol and ether.

RESULTS AND DISCUSSION

Infrared Spectrometry

The interpretation of the IR spectra of chitosan and its derivatives is to be found in recent papers by Muzzarelli *et al.* (1981*a*,*b*; 1982), Sannan *et al.* (1978), Galat & Popowicz (1978) and Moore & Roberts (1980).

The IR spectra recorded on *N*-(*o*-carboxybenzylidene) chitosan showed bands at 1600 and 1100 cm^{-1} (aromatic ring), 1580 cm^{-1} (double bond conjugated with the aromatic ring), 1645 cm^{-1} (C=N stretch) and 1690 cm^{-1} (carbonyl groups). Figure 1 shows the spectra of the NCBCs obtained upon reduction and insolubilisation at various pH values. In all five spectra, a band is present at 750 cm^{-1} and it is assigned to aromatic C—H. Aromatic *o*-substituted COOH present absorption bands around 1690 cm^{-1}. In our spectra in Fig. 1, absorption bands due to the amide groups are also present at this wave number and therefore the aromatic COOH band is not sharply distinguishable. In going from acidic to alkaline pH values, a remarkable increase in absorption is observed at 1550 and 1600 cm^{-1}, due to the carboxylate anion, with contributions from the amine functions. The IR spectra, therefore, confirm the presence of aromatic rings and carboxy groups in the NCBC preparations.

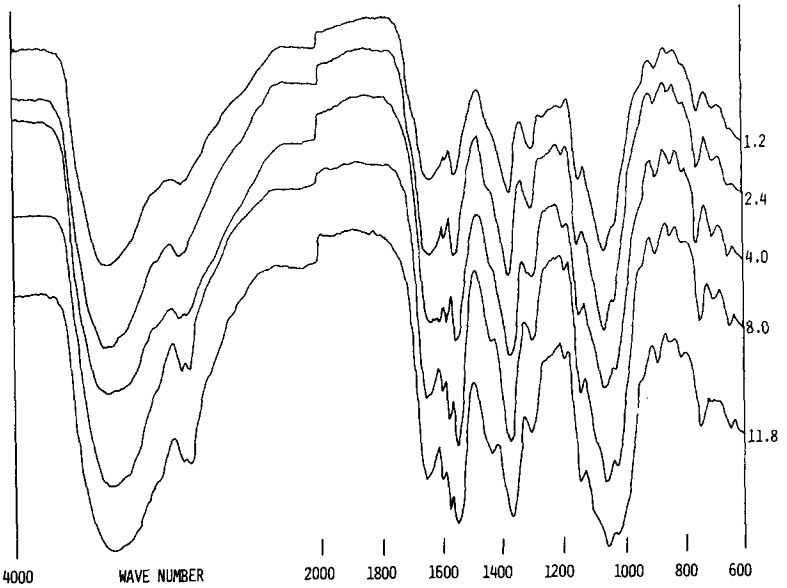

Fig. 1. IR spectra of NCBCs precipitated from aqueous solutions at various pH values (1·2, 2·4, 4·0, 8·0 and 11·8).

UV-Vis. Spectrophotometry

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 (spectrum (a) in Fig. 2). Phthalaldehydic acid solutions brought to pH 12·5 with NaOH present absorption maxima at 216·0, 252·0 and 292·0 nm (spectrum (b) in Fig. 2). NCBC solutions brought to pH 1·6 with HCl present absorption maxima at 204·0, 230·0 and 274·0 nm as shown by spectrum (c) in Fig. 2, while in alkaline solutions absorption occurs at 220 nm only.

The absorption band at 274·0 nm can be used to determine the degree of substitution of NCBC as well as the concentration of NCBC in solution.

Since the chitosan used was known to have a degree of deacetylation of 58 ± 4%, according to IR spectrometry, the NCBC, if fully carboxybenzylated, would have the same degree of carboxybenzylation (58 ± 4%) and secondary amine content (58 ± 4%). The spectrum of 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 assuming a 58% degree of carboxybenzylation, was compared with a 0·2% solution of NCBC. The degree of carboxybenzylation referred to the free amine available was then estimated as 74 ± 3%, that is to say 43 ± 3% of the total.

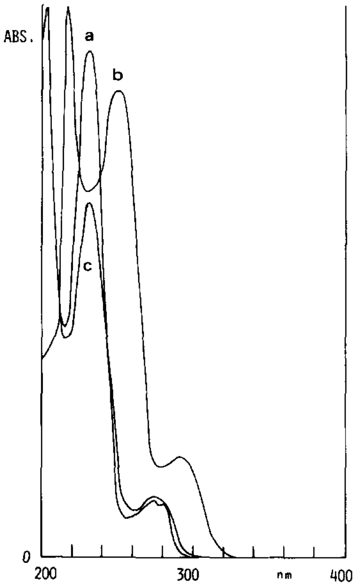

Fig. 2. UV-Vis. spectra of (a) phthalaldehydic acid at pH 1·6 (279 μM); (b) phthalaldehydic acid at pH 12·5 (279 μM); (c) NCBC at pH 1·6 (167 mg/litre).

The NCBC concentrations were 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 carried out in conjunction with the metal ion collection measurements described below.

Titration

From the above experimental data, the NCBC prepared possesses 42 ± 4% acetylated groups, 43 ± 3% carboxybenzylated groups (secondary amine) and 15 ± 1% free amine groups. Alkalimetric titrations of NCBC gave the curve displayed in Fig. 3. On this curve, the first inflection point (pH 3·9) is due to the complete titration of the carboxyl groups: at this point, the carboxyl group is completely dissociated (COO^-), while the amines are protonated ($R'R''NH_2^+$ and $R'NH_3^+$). The second inflection point (6·8) is attributable to the neutralisation of the primary amine or part of the secondary amine in such a way that equal numbers of negative and positive charges are present on the polysaccharide chain: it corresponds to the isoelectric point; in fact, it is in the middle of the precipitation range. The third inflection point (9·5) can be attributed to the complete neutralisation of the amine groups.

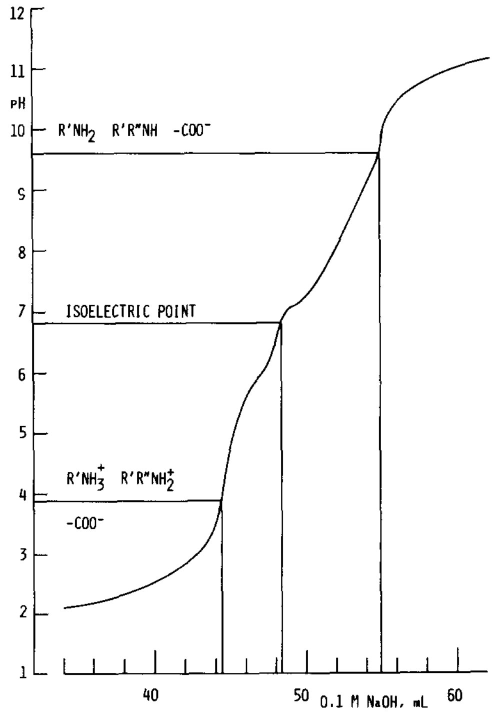

Fig. 3. Titration curve for NCBC obtained under nitrogen; precipitation is observed between pH 5·8 and 7·2.

To move from the 1st to the 2nd inflection points requires 4·0 ml 0·1 M NaOH while 6·6 ml is required to move from the 2nd to the 3rd. The sum of these increments is 10·6 ml 0·1 M NaOH which is necessary for the titration of the whole amine functionality. This, and preceding data correspond to a NCBC possessing the following characteristics: acetylation degree, 42 ± 4%; carboxybenzylation degree, 37 ± 3%; free amino groups, 21 ± 2%. The pK_a values were 5·7 and 8·0 (calculated at half titre) while the water content was 16% by weight.

Viscometry

The viscosity of the NCBC solutions was higher than that of chitosan though lower than that of NCMC. Thus, it was necessary to prepare 0·25% NCBC solutions for measurement under the same instrumental setting as used for 1·0–1·5% chitosan solutions.

As shown in Fig. 4, NCBC is thixotropic at pH 2·4 (as well as at 11·8); at the other pH values, thixotropy is nearly absent. Viscosity depends on pH, as illustrated in Fig. 5. At pH 4·0, the viscosity of NCBC solutions is at a maximum, in agreement with the titrimetric data which indicate pH 3·9 as the value at which maximum charge density exists on the polysaccharide chains.

Metal Ion Collection

One of the interesting properties of NCBC is its ability to chelate metal ions, thus yielding insoluble NCBC-metal chelates, which readily settle as precipitates or gel within minutes after mixing, depending on the reagent concentrations.

The insolubilisation of metal ions by chelation with soluble NCBC is pH dependent (Fig. 6). In the cases of Cr, Cu, Zn, Hg and Pb, maxima are observed at neutrality, while Co and Ni exhibit maxima at 8·5. In all cases, except with Cd and U, a sharp decrease in the collection percentage is observed at alkaline pH values. This fact would

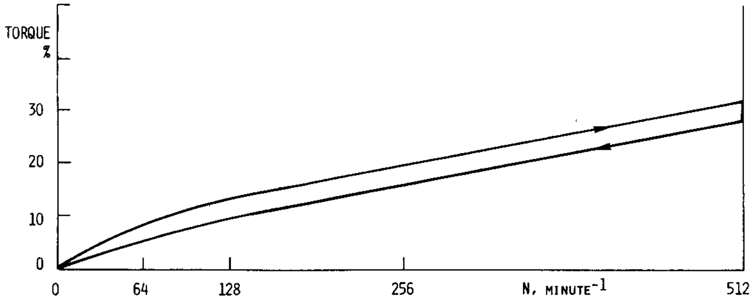

Fig. 4. Viscometry on a 0·25% NCBC solution at pH 2·4 and 25°C. The splitting of the curves upon shearing indicates thixotropy. Program 0·1–2·5–2·0 min. Upper curve, acceleration; lower curve, deceleration. (The torque will be proportional to shear stress and the rotation speed (*N* rpm) will be proportional to shear rate.)

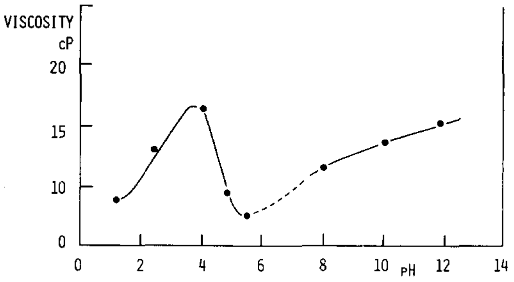

Fig. 5. Viscosity dependence on pH for a 0·25% NCBC solution at 25°C. Dotted line shows the partial insolubility interval.

indicate, first of all, that insolubilisation is not due to formation of insoluble hydroxides, which would mostly occur at pH 9 rather than 6–7, and that proper adjustments of pH permit the highest yields.

The collection percentages plotted in Fig. 7 have therefore been measured for solutions whose pH was within a restricted interval of values. Linear dependence of the collection percentages on the polymer concentration was observed in almost all cases even when low metal ion concentrations (0·1 mM) were studied. NCBC concentration (around 200–300 ppm) could scavenge Cr, Ni, Cu, Cd, Pb and Hg from their respective 0·1 mM solutions.

In the NCBC concentration interval studied Cr, Co, Ni, Cu, Cd, Pb and U could be scavenged completely from 0·1 and 0·2 mM solutions. Cr, Cu and Pb could also be completely removed even when present at higher concentrations (0·3, 0·4 and 0·5 mM). Very low results were obtained for Mn.

The NCBC concentrations left in solution after chelation and filtration were measured by UV–Vis. spectrophotometry. After the complete removal of the metal ions under the conditions adopted, some NCBC remains in solution; for instance, after the complete removal of Co with 400 ppm NCBC, 50 ppm of NCBC are found in solution. After complete removal of Ni and Cd with 300 ppm NCBC, 25 ppm and 10 ppm of NCBC remain in solution, respectively. Typical data are shown in Fig. 8. It can be seen that small amounts of NCBC (200 mg) used on a 1 litre solution lower the Cd concentration from 0·100 mM to 0·033 mM and when 300 mg NCBC are used, the Cd concentration is lowered to 0·010 mM and the NCBC left is only 13 ppm.

The molar ratios between carboxybenzyl units and metal ions were calculated from the data in Fig. 7 and found to be close to 3 for Co, Ni and Cd, around 2 for Pb and Hg and about 1·5 for Cu. The contribution of the residual free amino groups should not be forgotten as they could possibly play a role especially in the chelation of copper.

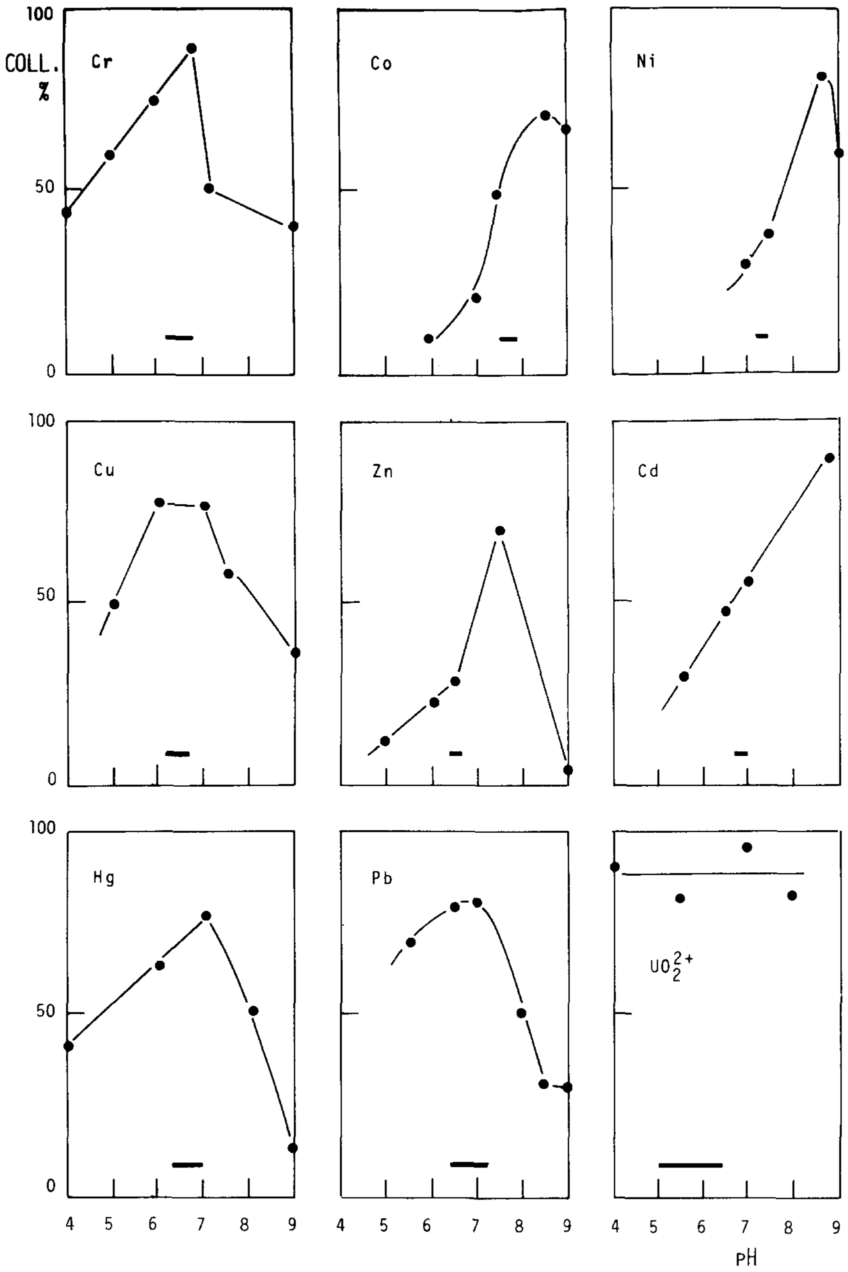

Fig. 6. Collection percentages of metal ions by NCBC as a function of pH. Bars indicate the pH intervals at which the data shown in Fig. 7 have been measured.

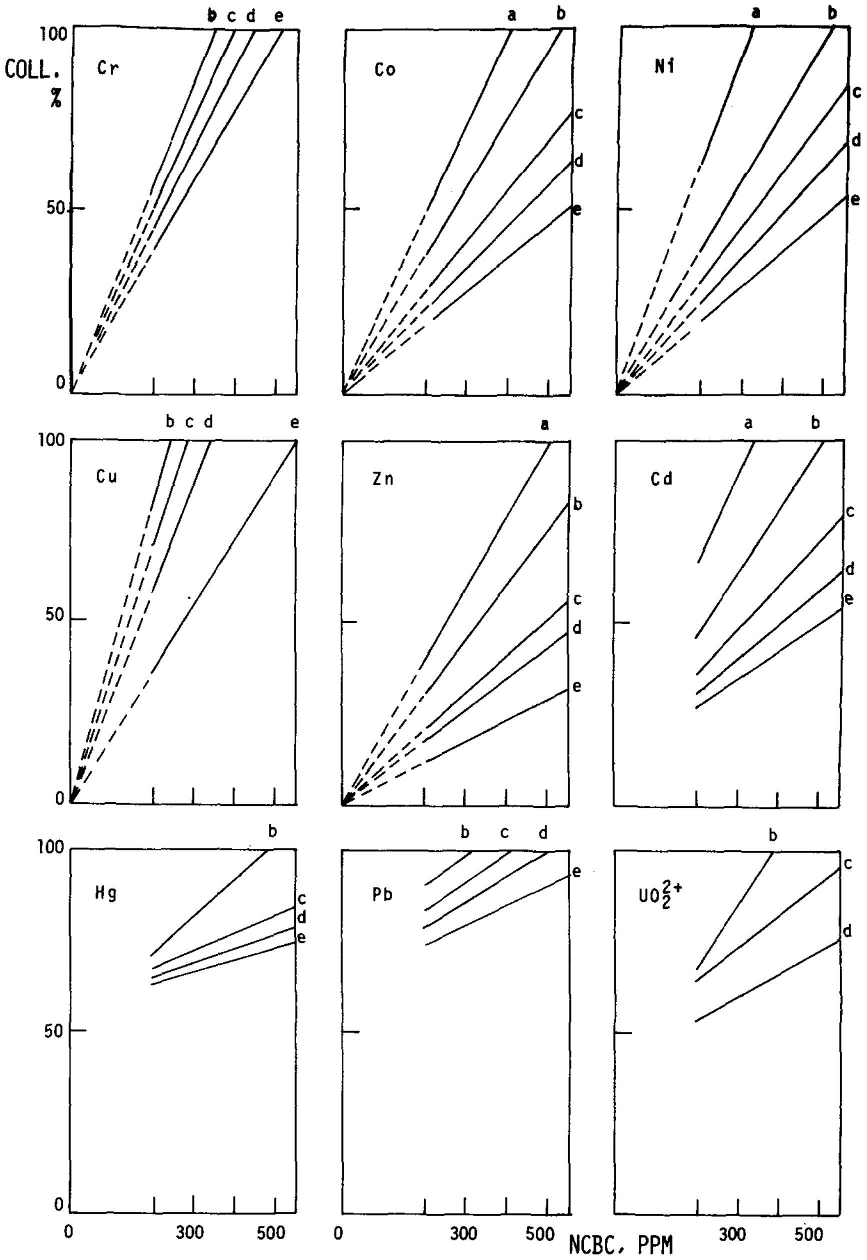

Fig. 7. Collection percentages of metal ions at various concentrations (a = 0·1, b = 0·2, c = 0·3, d = 0·4 and e = 0·5 mM) plotted as a function of NCBC concentration in ppm.

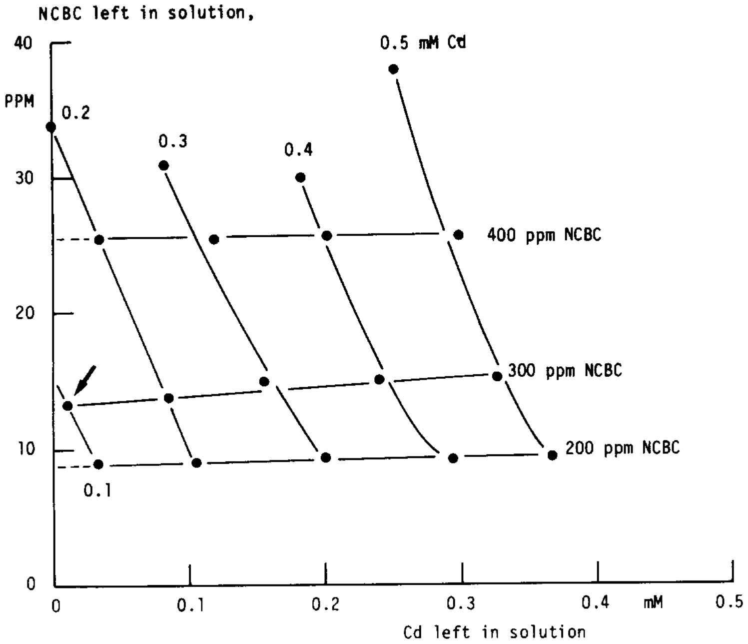

Fig. 8. NCBC left in solution (ppm) after chelation of Cd, against Cd left in solution (mM) at various NCBC and Cd concentration levels. For instance (arrow), when a 0·1 mM Cd solution is made to contain 300 ppm of NCBC, 90% Cd is removed and 13 ppm NCBC is left in solution.

CONCLUSIONS

N-(*o*-Carboxybenzyl) chitosans, a novel class of chelating polyampholytes, can be easily prepared under very mild conditions (aqueous media and room temperature) with common reagents (phthalaldehydic acid, chitosan and a reducing agent) within half a working day. NCBCs with degrees of substitution varied at will can be obtained by simply altering the molar ratios of the reagents.

The NCBC characterised in the present work carried a nearly equal number of acetylated and *o*-carboxybenzylated amino groups, together with a small amount of free amino groups. It should be emphasised that each *o*-carboxybenzylamino residue is simultaneously a secondary amine and a carboxylic acid. NCBC also contains a considerable amount of water which presumably is not simply absorbed, but is part of the polysaccharide structure, because it cannot be removed even after extraction with hydrophilic solvents.

NCBC powders are not hygroscopic, however, and they can be stored for weeks under normal laboratory conditions, without alteration. Once dissolved in water, however, NCBC cannot easily be recovered. Even at the isoelectric point, where the solutions become milky, no real coagulation takes place. Insolubilisation requires the addition of large quantities of organic solvents. It is obvious that such a polyampholyte exhibits different IR spectra depending on the degree of protonation. Therefore, the IR spectra of these products should always report the pH value at which the sample was isolated.

NCBC can easily be determined in solution by UV-Vis. spectrophotometry, using the absorption band due to the aromatic ring.

Chelation of metal ions leads to cross-linking, which explains sudden precipitation or gel formation. The molar ratios between NCBC and metal ions are close to 3 and 2, as expected on the basis of the bidentate nature of the *o*-carboxybenzylamino function. Thus, 6- and 4-coordinated complexes would involve donor atoms belonging to different functions and polymeric chains and probably also water and hydroxyl ions.

The NCBC performances for the chelation of metal ions can be optimised for applications in various fields, by taking advantage of the possibility of altering the degree of substitution, adjusting the pH and monitoring the NCBC concentration by spectroscopic means.

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