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N-(CARBOXYMETHYLIDENE)CHITOSANS AND *N*-(CARBOXYMETHYL)-CHITOSANS: NOVEL CHELATING POLYAMPHOLYTES OBTAINED FROM CHITOSAN GLYOXYLATE

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ABSTRACT

Glyoxylic acid, added to aqueous suspensions of chitosan, causes immediate dissolution of chitosan and gel formation within 3–4 h if the pH is 4.5–5.5. Solutions at lower pH values gel after 2 min of warming at 60–80°. Chitosan glyoxylate solutions brought to alkaline pH with sodium hydroxide do not precipitate chitosan. Evidence is given that a Schiff base, namely *N*-(carboxymethylidene)chitosan, is formed. *N*-(Carboxymethylidene)chitosans are reduced by sodium cyanoborohydride at room temperature to give *N*-(carboxymethyl)chitosans, obtained as white, free-flowing powders, soluble in water at all pH values. A series of *N*-(carboxymethyl)-chitosans having various degrees of acetylation and *N*-carboxymethylation was obtained, and characterized by viscometry, elemental analysis, and i.r. spectrometry. For the fully substituted *N*-(carboxymethyl)chitosans, the pK′ is 2.3, the pK″ is 6.6, and the isoelectric point is 4.1. The addition of *N*-(carboxymethyl)chitosan to solutions (0.2–0.5mM) of transition-metal ions produces immediate insolubilization of *N*-(carboxymethyl)chitosan–metal ion chelates.

INTRODUCTION

The carboxymethylation of chitin and chitosan has thus far been conducted by treating "alkali chitin" with monochloroacetic acid, and has been therefore directed to the alcoholic groups only[1]. The preparation of *O*-(carboxymethyl)chitosan included swelling of chitin in dimethyl sulfoxide, treatment with 65% sodium hydroxide, reaction with monochloroacetic acid, and deacetylation in hot sodium hydroxide. As monochloroacetate decomposes in alkali, the preparation is possible only because the rate of carboxymethylation is 20 times as great as the velocity of the side reaction. As this preparation includes two steps of treatment with sodium hydroxide, small variations in the experimental conditions lead to polymers having different properties, such as water solubilities, degrees of deacetylation, and molecular sizes; furthermore, the final product is a sodium salt. *O*-(Carboxymethyl)chitosan mixed with chitosan has been used to remove Cu^{2+} from waste water[2].

In the past, we have proposed the use of chitosan solutions as precipitating

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agents for the removal of metal ions from industrial and sea water[3]. We have also reported data on the chelation ability of alginic acid, D-galacturonan, and other carboxylated polysaccharides[4], and these have been confirmed by subsequent results[5,6]. We have considered that a polymer bearing both functions (amine and carboxyl) would possess the most desirable properties for the rapid and effective chelation and insolubilization of transition-metal ions in water[1]. Preparation of such a polymer would need to be simpler than the *O*-(carboxymethyl)chitosan just described, and should have a better defined nature than various chitosan complexes incorporating *O*-(carboxymethyl)dextran, *O*-(carboxymethyl)cellulose, heparin, and other acidic polysaccharides[7,8].

Acetic acid, oxalic acid, and dichloroacetic acid, show significant interactions with chitosan[1]: acetic acid is the most widely used solvent for this biopolymer: dichloroacetic acid possesses peculiar characteristics not matched by mono- and trichloroacetic acids as a solvent for special applications[9] and has been used to synthesize photosensitive polymers from chitosan[10]; and oxalic acid is unusual in that it forms non-Newtonian solutions with chitosan, which become thermoreversible gels[11]. These three molecules possess in common two carbon atoms, one or both being linked to oxygen (carbonyl group) or to two chlorine atoms. A similar molecule possessing both carbon atoms in the carbonyl form is glyoxylic acid (HO_2CCHO). Its chemistry offers analogies with that of dichloroacetic acid and methyl dichloroacetate; glyoxylic acid is used in organic synthesis, especially in the pharmaceutical field.

Our original approach to the carboxymethylation of chitosan[12] consisted of reacting the free amino groups of chitosan with glyoxylic acid to produce a soluble, gel-forming imine[13] and then reducing the product with a suitable reducing agent, such as cyanoborohydride. This preparation is very readily effected, does not require warming or cooling, and requires only commercially available reagents. Thus produced is a variety of *N*-(carboxymethyl)chitosans, containing acetyl, carboxymethyl, and free amino groups in proportions readily controlled through the choice of the starting chitosan (degree of acetylation and molecular weight) and the amount of glyoxylic acid used.

Chitosans modified through reactions at the free amino groups have been described in a number of papers; such examples include Schiff bases with glutaraldehyde and similar reagents[14]; *N*-acylchitosans[15,16]; *N*-alkylidenechitosans[17]; and branched-chain chitosans obtained from monosaccharides[18]. This report is the first on a derivative of chitosan carrying a carboxyl group on the amine function.

EXPERIMENTAL

Reagents. — Chitosan from *Euphausia superba* (antarctic krill), degree of deacetylation 58 ±4%, was supplied by Rybex, Szczeszyn, Poland. Glyoxylic acid monohydrate and sodium cyanoborohydride were supplied by Merck–Schuchardt.

Preparation of N-(*carboxymethylidene*)*chitosans.* — A sufficient quantity of

glyoxylic acid is added to an aqueous suspension of chitosan to cause immediate dissolution, yielding a clear solution that is more or less viscous according to the concentration of biopolymer. The resulting pH is 3.2 when sufficient glyoxylic acid (0.57 g) is added to the chitosan powder (1 g) suspended in water (30 mL) to give a molar ratio 1.0 between glyoxylic acid and 2-amino-2-deoxy-D-glucose residues. Similar solutions may be obtained when the molar ratio of chitosan to glyoxylic acid is < 1.0. The pH values of these solutions are established by the glyoxylic acid present, but they do not explain the sudden and complete dissolution of chitosan. Other acids, stronger or variously substituted, do not possess the ability to dissolve chitosan, as has been widely reported.

When M sodium hydroxide was added to stirred solutions of chitosan in glyoxylic acid, the solutions became milky near neutral pH, but further addition of an excess of M sodium hydroxide (pH ~ 12) restored clarity. For dissolution of chitosan at pH 12, the following recommendations should be strictly followed: up to pH 4.5, the M sodium hydroxide should be added slowly, with pauses if necessary between pH 3.2 and 4.5, to avoid the formation of a precipitate. As soon as the solution becomes milky, the alkali has to be added very rapidly in order to reach pH 12 within a few sec. Different procedures may lead to turbid, alkaline solutions.

The foregoing facts indicate that chitosan reacts with glyoxylic acid to produce a polyampholyte soluble in alkaline media in which chitosan is insoluble. The solutions of chitosan in glyoxylic acid (molar ratio chitosan/glyoxylic acid ≤ 1.0) at various pH values and at room temperature form gels shortly after their preparation; gel formation occurs at once upon heating.

The following scheme is therefore proposed for the reaction of chitosan and glyoxylic acid:

$$\underset{\substack{\text{Chitosan glyoxylate,}\\ \text{pH 3.2,}\\ \text{soluble}}}{OHCCO_2^- \ {}^+H_3N\text{-Chit}} \rightleftharpoons \underset{\substack{\text{Chitosan imine,}\\ \text{neutral pH,}\\ \text{insoluble}}}{\text{Chit-N=CHCO}_2\text{H}} \rightleftharpoons \underset{\substack{\text{Chitosan imine sodium salt,}\\ \text{alkaline pH,}\\ \text{soluble}}}{\text{Chit-N=CHCO}_2^- \ Na^+}$$

When the pH is favorable for formation of the imine (pH 4.5, 5.0), the latter is formed readily at room temperature. When the pH is less favorable (pH 3.2, 3.7) gels form at higher temperature: the temperature for gel formation depends almost linearly upon pH.

The increase of viscosity with temperature is attributable to the increase in molecular weight through the condensation reaction and to the consequent intermolecular and intramolecular interactions between oppositely charged groups.

The concentration of chitosan is a limiting factor in gel formation. Solutions of chitosan (molar ratio 1.0, pH 4.5) form gels at 80° only when the concentration reaches 16 g/L and at 70–80° when the concentration reaches 25 g/L. At pH 4.5, the concentration of chitosan needs to be at least 33 g/L if gels are to be formed at 20°.

Preparation of N-(*carboxymethyl*)*chitosans*. — The required amount of sodium

cyanoborohydride dissolved in a limited amount of water was added to the solutions of *N*-(carboxymethylidene)chitosan with stirring; the pH rose to 6–7. A few sec after completing the addition, the viscosity of the solution increased to the extent that magnetic stirring was no longer possible. Ethanol was used to insolubilize and wash the product, obtained as a white, free-flowing powder. The samples, washed with fresh ethanol, were then lyophilized at −40° and 0.1 torr, overnight. For the purpose of i.r. spectroscopy, aliquots of *N*-(carboxymethyl)chitosans were reprecipitated with acetone and washed with ethanol, from solutions at pH 1 and 12; these were the hydrochloride and sodium salts, respectively. Four preparations were made, all with yields >90%:

(*a*) Chitosan (10 g) in water (2 L) was dissolved with glyoxylic acid (5.75 g) and reduced with $NaBH_3CN$ (4 g) at pH from 4.0–6.3.

(*b*) The same as (*a*) but with less water (1.3 L) and a longer period of time (4 days) between addition of $NaBH_3CN$ and insolubilization. For these two preparations, the amount of glyoxylic acid used was 185% of the stoichiometric, to ensure full substitution.

(*c*) Chitosan (5 g) in water (700 mL) was dissolved with glyoxylic acid (0.776) and hydrochloric acid (2 mL, conc.), and then reduced with $NaBH_3CN$ (0.54 g) at pH 5.0–6.3, and then insolubilized 3 h later.

(*d*) Chitosan (5 g) in water (700 mL) was dissolved with glyoxylic acid (0.388 g) and hydrochloric acid (2 mL, conc.), reduced with $NaBH_3CN$ (0.27 g) at pH 5, and then adjusted to 6.0 with sodium hydroxide, and insolubilized 3 h later.

Viscometry. — Measurements were made with a Haake Rotovisco RV 12 viscometer, equipped with a programmer, a model SV1 rotor, and a Hewlett–Packard recorder; the temperature was controlled with a Haake thermostat–cryostat. The readings (see Fig. 7) were plots of torque S versus test speed n. Factors (M, A, and G) necessary for calculating shear rate, shear stress, and viscosity were: $A = 12.4$ Pa/scale gradation; $M = 0.89$ min/s, and $G = 13920$ mPa s/scale gradation min. The shear rate was calculated as $D = M \cdot n$ (s^{-1}); shear stress as $\tau = A \cdot S$ (Pa) and viscosity as $\eta = G \cdot S/n$ (mPa · s). The conversion of viscosity data from S.I. into c.g.s. units was as follows: viscosity, η: pascal · second (Pa · s); 1 Pa · s = 10 poise; shear stress τ: pascal (Pa), 1 Pa = 10 dyne · cm^{-2}; shear rate D: reciprocal second (1/s). The sample volume was 12 mL.

Infrared spectrometry. — Chitosan powders were ground with i.r.-grade potassium bromide in an agate mortar. Spectra were recorded with a Perkin–Elmer infrared spectrometer Model 299-B, on translucent discs obtained by pressing the ground material with the aid of a Perkin–Elmer press.

Atomic absorption spectrometry. — Analyses were performed with a P.E. 305 spectrometer, equipped with flame and graphite atomizers, according to standard methods.

Alkalimetry. — An AMEL pH meter was used in conjunction with Titrisol 0.1M hydrochloric acid and sodium hydroxide solutions. The samples of *N*-(carboxymethyl)chitosan (0.5 g) were dissolved in 0.1M hydrochloric acid (50 mL) and titrated

with 0.1M sodium hydroxide under nitrogen. Another set of data was obtained by using both 0.1M hydrochloric acid and 0.1M sodium hydroxide made to contain 0.1M sodium chloride. Additions were made at time intervals to permit accurate readings, especially in the central part of the pH range.

Metal-ion chelation and insolubilization. — Metal sulfate solutions (0.2, 0.3, 0.4, and 0.5mM) contained 10, 15, 20, and 25 mg of *N*-(carboxymethyl)chitosan in 50 mL. They were agitated on a shaking machine at 80 r.p.m. for 1 h, and then filtered on Whatman filter paper and analyzed by atomic absorption spectrometry.

RESULTS AND DISCUSSION

Infrared spectrometry. — Assignment and interpretation of the bands were made

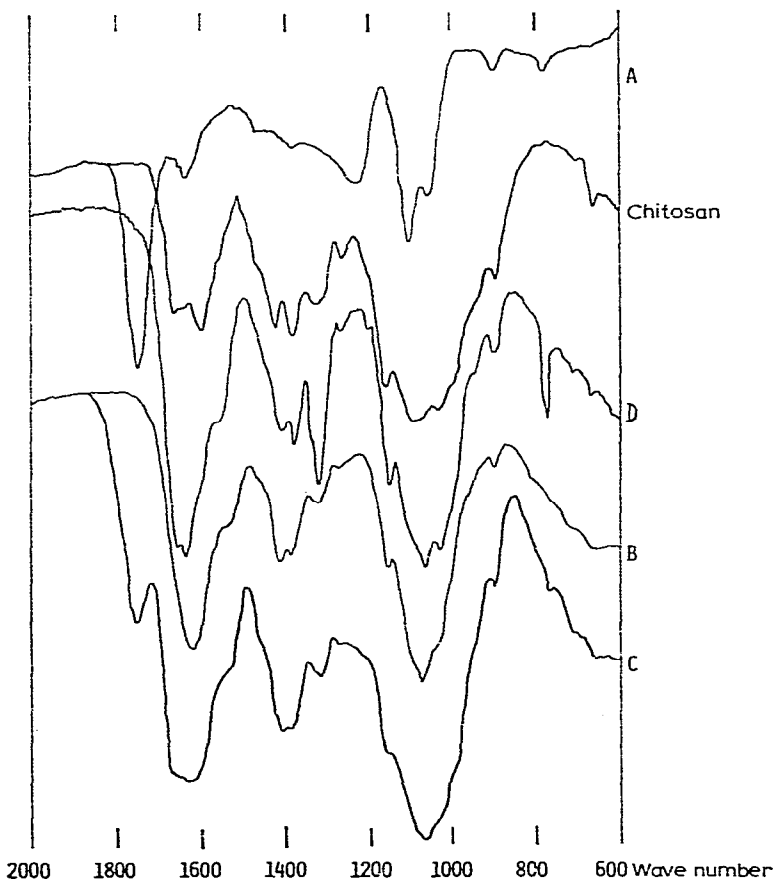


Fig. 1. I.r. spectra of (A) glyoxylic acid monohydrate; (B) gel obtained from chitosan and glyoxylic acid at 20°, pH 5.0, molar ratio 1.0, *N*-(carboxymethylidene)chitosan; (C) product obtained after heating a mixture of chitosan and glyoxylic acid powders for 3 h at 120°, molar ratio 1.0; and (D) precipitate obtained after addition of ethanol to the alkaline solution of *N*-(carboxymethylidene)-chitosan.

in the light of recent investigations on chitin and chitosan by ourselves[19] and others[20–23]. Fig. 1 shows the i.r. spectrum of *N*-(carboxymethylidene)chitosan taken on the gel obtained at room temperature (molar ratio 1.0) after washing with acetone to remove excess glyoxylic acid; it is sharply different from the spectrum of glyoxylic acid and significantly different from the i.r. spectrum of chitosan. The bands at 1620–1630 and 1420 cm^{-1} correspond to substituted amines and imines and to the carboxyl ion, respectively. At 1580 cm^{-1}, a further contribution of the carboxyl ion (internal salt) is also evident. These bands become more evident with decreasing molar ratios in the sample preparation. Fig. 2 shows the i.r. spectra of fully substituted samples of *N*-(carboxymethyl)chitosan obtained at various pH values: 2.2, 2.7, 6.5, and 12. The 1730-cm^{-1} band (CO_2H) appears at pH 2.7 and is clearly present at pH 2.2, whereas at higher pH values it is absent. Proceeding from pH 2.2 to 12, the band at 1730 cm^{-1} decreases whereas those at 1580 [ν_{as} (CO_2)] and 1400 [ν_{sym} (CO_2)] increase as a consequence of the ionization of CO_2H to CO_2^-, particularly above pH 2.7 where the polymer is in the form Chit-$N^+H_2CH_2CO_2^-$. Fig. 3 shows the i.r. spectra of the variously substituted polymers insolubilized at pH 1: there, the band at 1730 cm^{-1} (CO_2H) increases as the degree of substitution increases. It also appears that the absorption at 1500 cm^{-1} (NH_3^+) decreases on progressing from chitosan

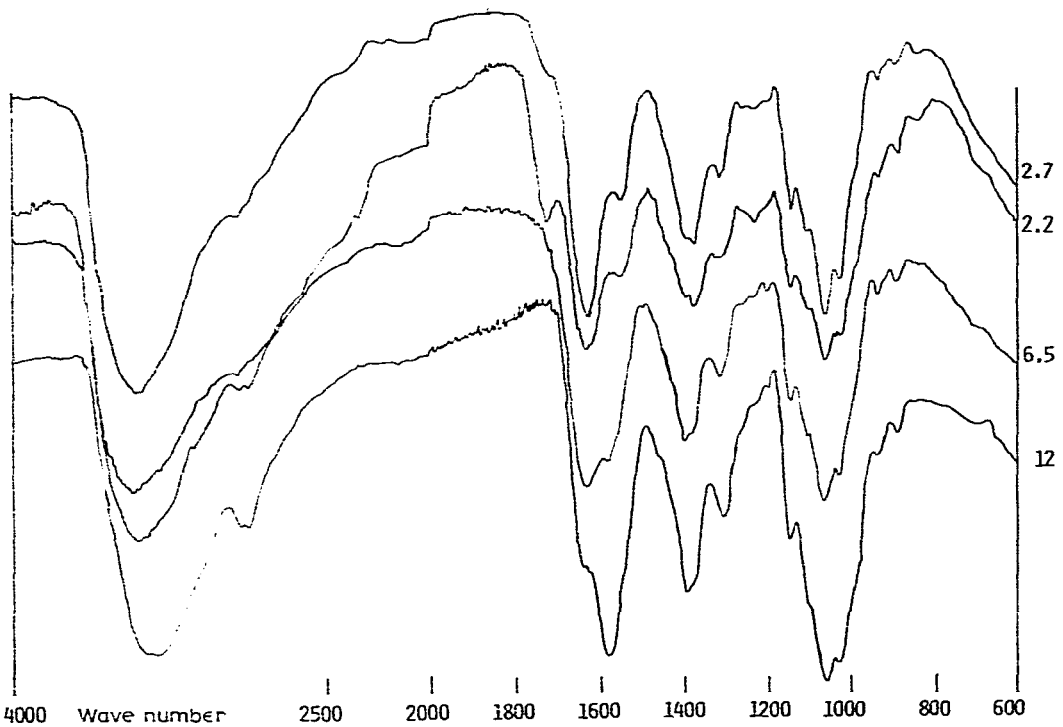


Fig. 2. I.r. spectra of fully carboxymethylated (58%) *N*-(carboxymethyl)chitosans insolubilized at various pH values (hydrochloride and sodium salt) showing the effects of pH on the bands at 1730, 1580, 1500, and 1400 cm^{-1}.

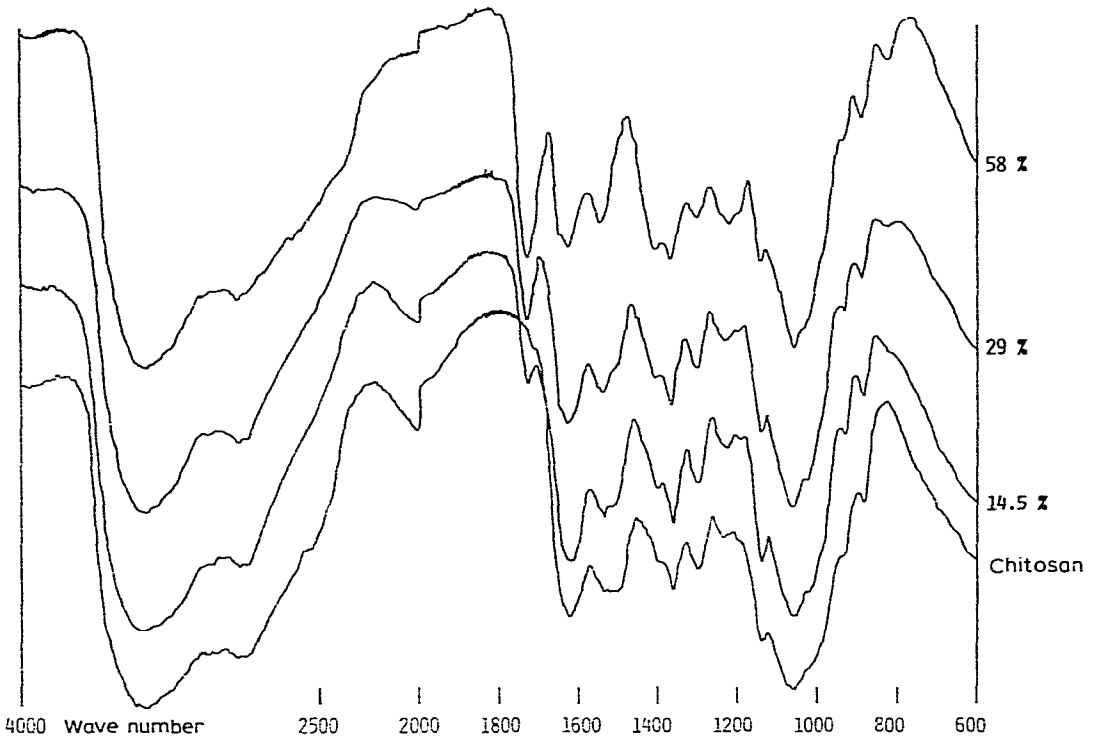


Fig. 3. I.r. spectra of *N*-(carboxymethyl)chitosans having various degrees of carboxymethylation, insolubilized at pH ~ 1 (hydrochlorides) showing the effects of the degree of carboxymethylation on the bands at 1730, 1580, 1500, and 1400 cm^{-1}.

(pH 1) to the fully substituted *N*-(carboxymethyl)chitosan. Fig. 4 shows the i.r. spectra of the same polymers as in Fig. 3, but insolubilized at pH 12. The absorption at 1580 cm^{-1} (CO_2^-) clearly increases in proceeding from chitosan to the fully substituted *N*-(carboxymethyl)chitosan. Less dramatic, but clearly visible, is the same trend at 1400 cm^{-1}, ν_{sym} (CO_2). If the spectra in Fig. 4 are compared with those taken on the polymer isolated at pH 12 before reduction (Fig. 1, curve D), one of the most significant differences is the absence in the former of the band at 770 cm^{-1} (out-of-plane deformation of the $N{=}CHCO_2^-$ group): its absence is a consequence of the reduction of the N=C double bond.

Elemental analysis and differential thermal analysis. — Differential thermal analysis gave absolutely flat recordings on all samples over the interval 30–150°. Elemental analysis confirmed the degree of deacetylation of chitosan [55% by elemental analysis; 59% according to alkalimetric data in Fig. 6 (Ref. 1 p. 105), and 58 ±4% by i.r. spectrometry[19]]. The results were: C, 42.2; H, 6.87, and N, 6.93%; calc. moisture content 9–10%. The elemental analysis of the less-substituted sample (Type *d*) of *N*-(carboxymethyl)chitosan gave: C, 38.51; H, 6.90; and N, 5.70%. These experimental data correspond to an *N*-(carboxymethylated)chitosan having

42% of the amino groups acetylated, 14.5% *N*-carboxymethylated, and 43.5% free; the moisture content was 16%. According to a computerized search, based on the following weights for the repeating units: 2-amino-2-deoxy-D-glucose, 161.16; 2-acetamido-2-deoxy-D-glucose, 203.19; and 2-(carboxymethyl)amino-2-deoxy-D-glucose, 219.19, the best fit for all analytical data was for a class of polymers containing 16% moisture, and having a degree of deacetylation of 54–56% (Fig. 5). Interestingly, *O*-(carboxymethyl) chitosan was also reported to contain 16% of moisture (ref. 1, p. 121). Chitosan itself contains >10% moisture at 50% relative humidity[26]. The percentage of amino groups in the carboxymethyl form is 13.5%, that is, the value expected on the basis of the stoichiometric amount of glyoxylic acid used. Elemental analysis of the other samples (Types *a*, *b* and *c*) was difficult, presumably because of the absorption of gases, moisture, and solvents used for insolubilization, as well as difficulties experienced in washing. Nevertheless, if the analytical data are related to the corresponding point in Fig. 4, and it is assumed that the fully substituted *N*-(carboxymethyl)chitosan (42% acetylated and 58% carboxymethylated, ±4%) no longer has free amino groups (reasonable in view of the i.r. data and the excess of glyoxylic acid used in preparing it—see "Alkalimetry"), the linearity observed indicates sample Type *c* as an *N*-(carboxymethyl)chitosan having 42% acetylated, 29% carboxymethylated, and 29% free amino groups. The equimolar amounts of carboxyl

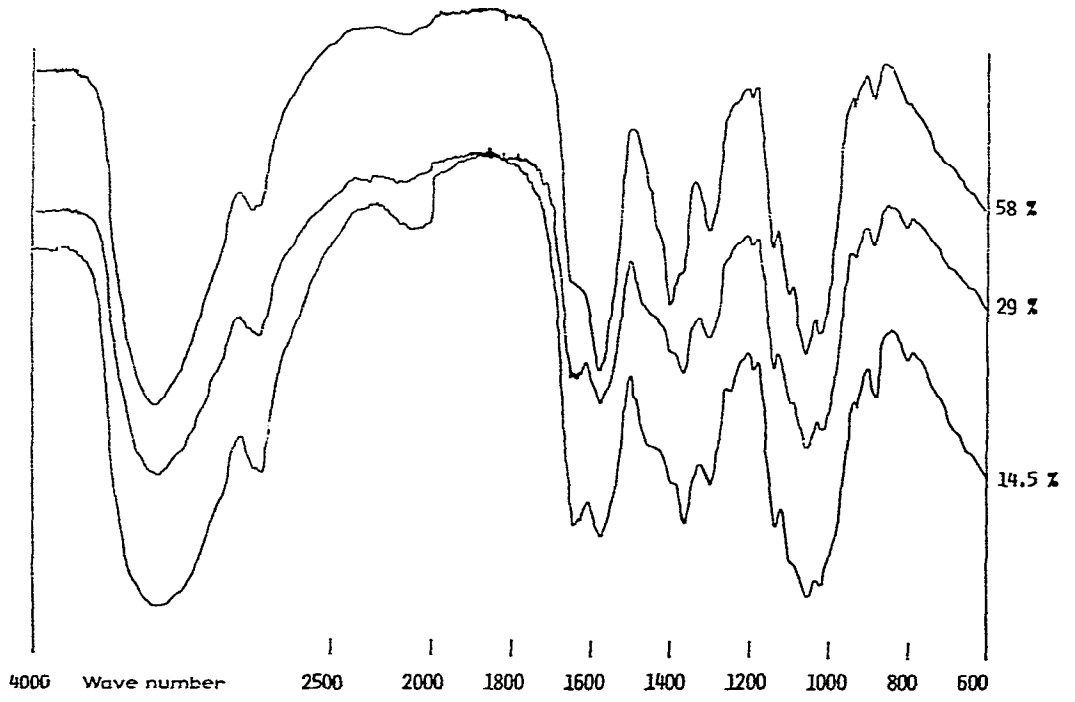
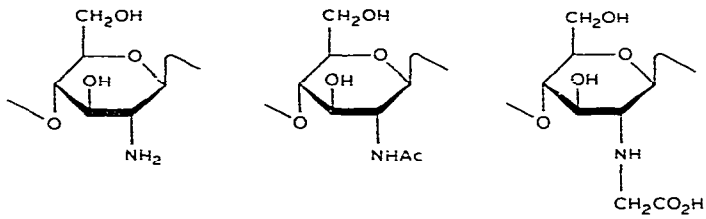


Fig. 4. I.r. spectra of *N*-(carboxymethyl)chitosans having various degrees of carboxymethylation, insolubilized at pH 12 (sodium salt) showing the effects of the degree of carboxymethylation on the bands at 1730, 1580, 1500, and 1400 cm^{-1}.



Mol. wt.: 161.15	203.19	219.19
Percent: 42 ±4	44 ±4	14 ±1
Av. Mol. wt.:	187.77	
Water content: 16%		

Anal. Calc. C, 38.47; H, 7.27; N, 6.26

Found: C, 38.51; H, 6.90; N, 5.90

Fig. 5. Description of a sample of *N*-(carboxymethyl)chitosan having degree of acetylation 44 ±4% and degree of carboxymethylation 14 ±1%, water content 16%.

and free amino groups are experimentally manifested by the highly fibrous nature of the dry product and higher viscosity of its freshly prepared solutions.

Alkalimetry. — The titration curve obtained with fully substituted *N*-(carboxymethyl)chitosans is shown in Fig. 6, which shows the neutralization points for each ionized group, and the pK values. The inflection points, in the presence of sodium chloride, were 2.0, 2.3, 4.1, 6.6, and 9.5 in the curve for *N*-(carboxymethyl)chitosan; the first one arises from the titration of hydrochloric acid in the presence of a fully protonated polymer; the second one corresponds to the pK_a; the third one to the titration of the carboxyl group of the polymer, which at pH 4.1 (isoelectric point) is $N^+H_2CH_2CO_2^-$, as confirmed by the i.r. spectra (Fig. 2); the fourth one is the pK_a, and the fifth one corresponds[27] to the complete titration to $NHCH_2CO_2^-$.

The samples treated with an excess of glyoxylic acid were found to be fully carboxymethylated, to the extent allowed by their degrees of deacetylation: (degree of carboxymethylation: 0.58).

Viscometry. — The variations of viscosity as a function of the temperature at which the samples of *N*-(carboxymethylidene)chitosan were brought for 2 min were studied. The viscosity increases sharply in those samples brought to temperatures >75° (pH 3.2) or >60° (pH 3.7), leading to transparent, light-yellow, and self-sustaining gels. Maximum viscosity was reached 75 min after the 2-min warming period. Solutions at pH values between 4.5 and 5.0 yield gels at room temperature (20°) without any warming. All gels absorbed large amounts of water or methanol and swelled enormously; white xerogels could be obtained after dialysis and lyophylization.

The viscosity of solutions of *N*-(carboxymethyl)chitosan was exceptionally high. Whereas chitosan is usually read on 1.0 or 1.5% solutions, it was necessary to prepare 0.2% solutions of *N*-(carboxymethyl)chitosan; one of the viscosity recordings on a 0.5% solution of *N*-(carboxymethyl)chitosan is shown in Fig. 7: in comparison

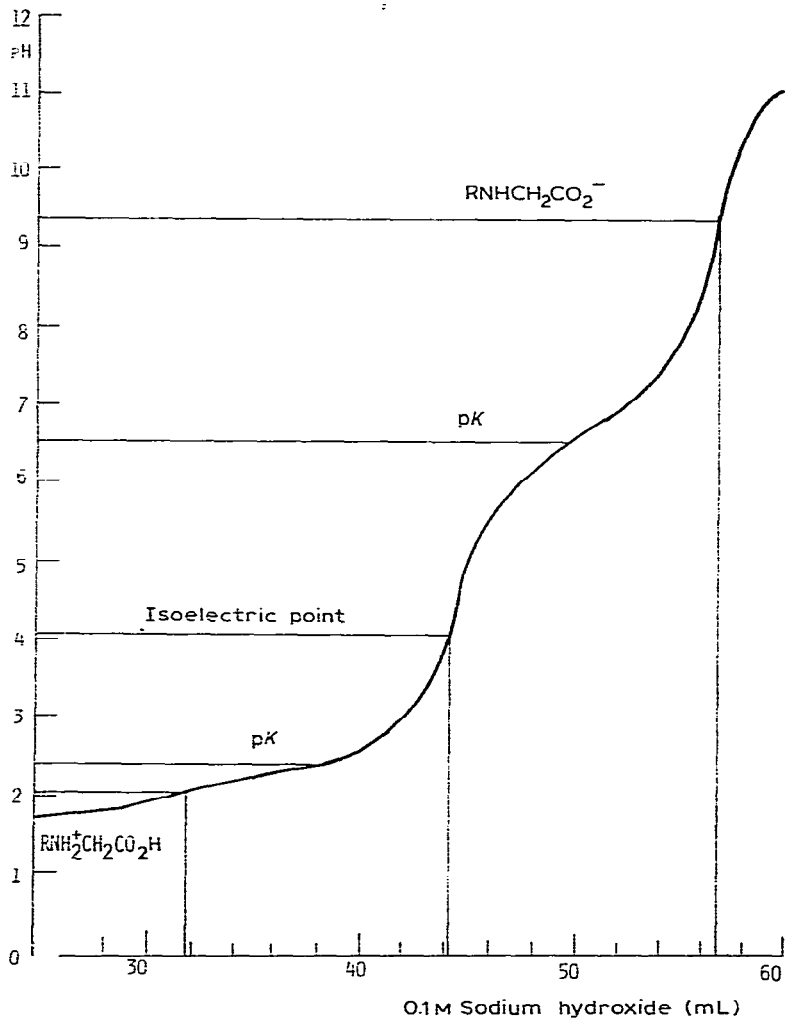


Fig. 6. Alkalimetric curve recorded on fully substituted *N*-(carboxymethyl)chitosan (sample *a*, having degree of carboxymethylation 58%) (0.5 g) in 0.1M HCl (50 mL), at 25° under nitrogen. The corresponding i.r. spectra are shown in Fig. 2.

to the readings taken on the chitosan of the same origin (1.5% solutions), *N*-(carboxymethyl)chitosan was much more viscous (330 Pa · s ≡ cPoises instead of 206) at 512 r.p.m. It proved impossible to record a viscosity curve at 1.5% concentration, because of excessive thickness, retention of air bubbles, and handling difficulties. The shape of the curve in Fig. 7 shows that *N*-(carboxymethyl)chitosan is a rheopessic (antithixotropic) product. The pH dependence of viscosity (Fig. 8) shows a behavior typical of polyampholytes[24].

A decrease in viscosity took place during the initial 48 h after dissolution, and then no further decrease was observed over a one-week period. The 29% substituted *N*-(carboxymethyl)chitosan initially showed higher viscosity (152 Pa.s) than the

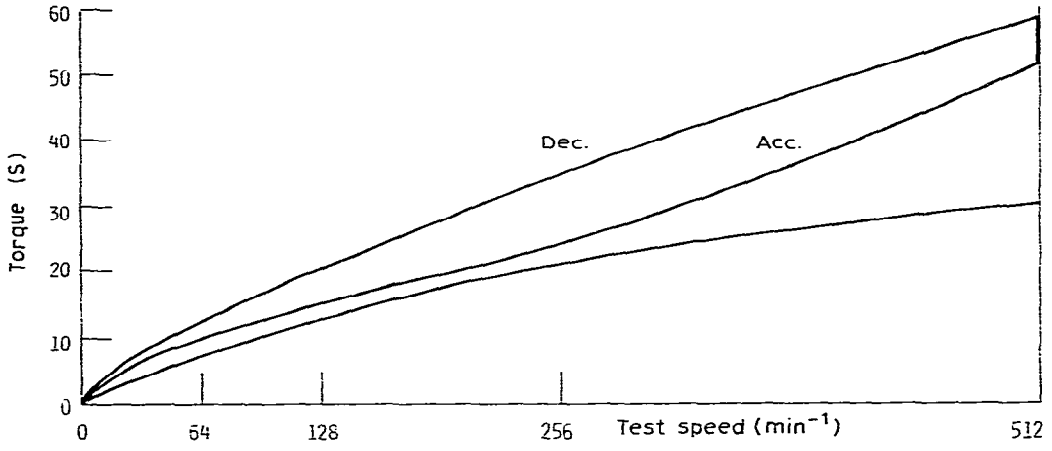


Fig. 7. Viscometry on a solution (0.5%) of fully substituted *N*-(carboxymethyl)chitosan, pH 3.4 at 25° (upper curves, deceleration and acceleration, program 0.1, 2.5, and 2.0 min), and on a solution (0.5%) of *N*-(carboxymethylidene)chitosan, pH 6.3 at 25° (lower curve coincident for acceleration and deceleration).

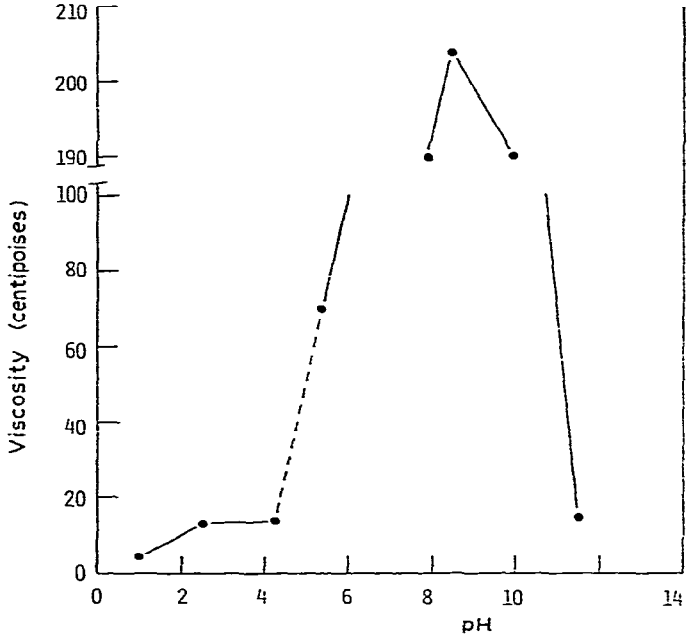


Fig. 8. Viscosity dependence on pH for a 0.25% (fully substituted) solution of *N*-(carboxymethyl)-chitosan at 25°. The dotted line covers the interval of partial insolubility.

other samples (66–85 Pa.s) at 0.2% concentration, with values on the sixth day in the same range (90–102 Pa.s for all samples, exception for sample type *b*, having 41 Pa.s). The standard deviation for these measurements was ±9%.

Differences between N-(*carboxymethyl*)*chitosan and the* N-(*carboxymethylidene*)-*chitosan from which it was obtained.* — Whereas gels could be obtained by adding amines to the products formed by the reaction of chitosan with glyoxylic acid, the same amines did not produce any precipitation or form gels when added to solutions of *N*-(carboxylmethyl)chitosan.

For preparative purposes, insolubilization may be performed at any pH value with *N*-(carboxymethyl)chitosan, whereas only alkaline pH values were suitable for insolubilization of *N*-(carboxymethylidene)chitosans. Viscosity and metal-binding data are other points of difference between the chitosan imine and *N*-(carboxylmethyl)-chitosan.

Metal-ion binding. — Chemical evidence for the presence of *N*-(carboxymethylidene)chitosan in solution and of its complexing ability is given by the altered classical chemistry of transition-metal ions. The presence of the polymeric aldimine in sodium hydroxide solutions hinders the precipitation of metal hydroxides: for instance, when a solution of copper(II) sulfate is added to an alkaline (pH 12) solution of *N*-(carboxymethylene)chitosan, a blue gel is formed immediately: it loses water afterwards and yields a violet precipitate. The precipitation of oxides, such as the well-known precipitation of yellow mercuric oxide from alkaline solutions of mercury(II) salts, is also hindered: in fact, no precipitate is observed if the alkaline solution contains *N*-(carboxymethylidene)chitosan.

One of the characteristic properties of *N*-(carboxymethyl)chitosan is its ability to chelate transition-metal ions, thus yielding insoluble *N*-(carboxylmethyl)chitosan–metal-ion chelates, which readily settle as hydrated solids within minutes after mixing. Even relatively dilute solutions (0.2mM) may be conveniently treated with solutions of *N*-(carboxymethyl)chitosan to collect transition-metal ions: the supernatant solution may be removed by paper filtration or centrifugation.

The fully substituted *N*-(carboxymethyl)chitosan were particularly suitable for precipitating transition-metal ions; less-substituted *N*-(carboxymethyl)chitosans formed precipitates that did not settle quickly and remained gelatinous. Figs. 9 and 10 report data for the binding of nine metal ions by the fully substituted *N*-(carboxymethyl)chitosan (sample *b*).

The insolubilization of metal ions by chelation with soluble *N*-(carboxymethyl)-chitosan is pH dependent (Fig. 9). With Co, Cu, Zn, Hg, and Pb, maxima are observed at neutrality (pH 6–7), whereas Ni and Cd show maxima at 7.5 and U at 5.5. The proper adjustment of pH permits attainment of the highest yields. The collection percentages plotted in Fig. 10 were therefore measured with solutions whose pH was within a restricted interval. A linear dependence of the binding percentages on the concentration of *N*-(carboxymethyl)chitosan was observed in almost all instances, even with low metal-ion concentration (0.2mM). In the concentration interval studied, *N*-(carboxymethyl)chitosan completely scavenged Co, Ni, Cu, Cd, Pb, and U from 0.2mM solutions; Cu, Hg, Pb, and U could also be completely removed, even when present at higher concentrations (0.3–0.5mM).

If the average molecular weight of the repeating unit for the fully substituted

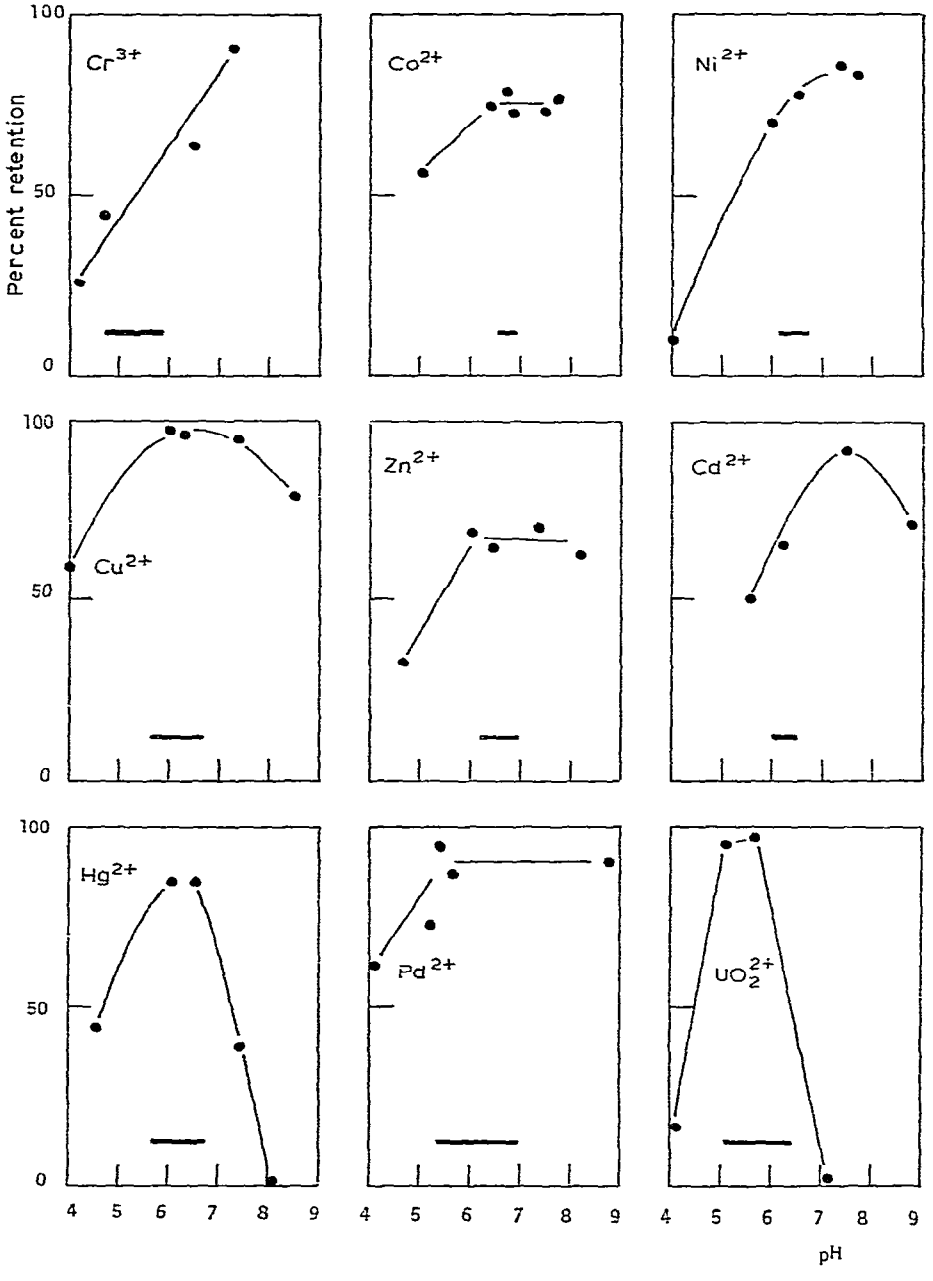


Fig. 9. Percentages of collection for metal ions on *N*-(carboxymethyl)chitosan (fully substituted) *vs* pH value. Bars indicate the pH intervals at which the data of Fig. 7 were measured.

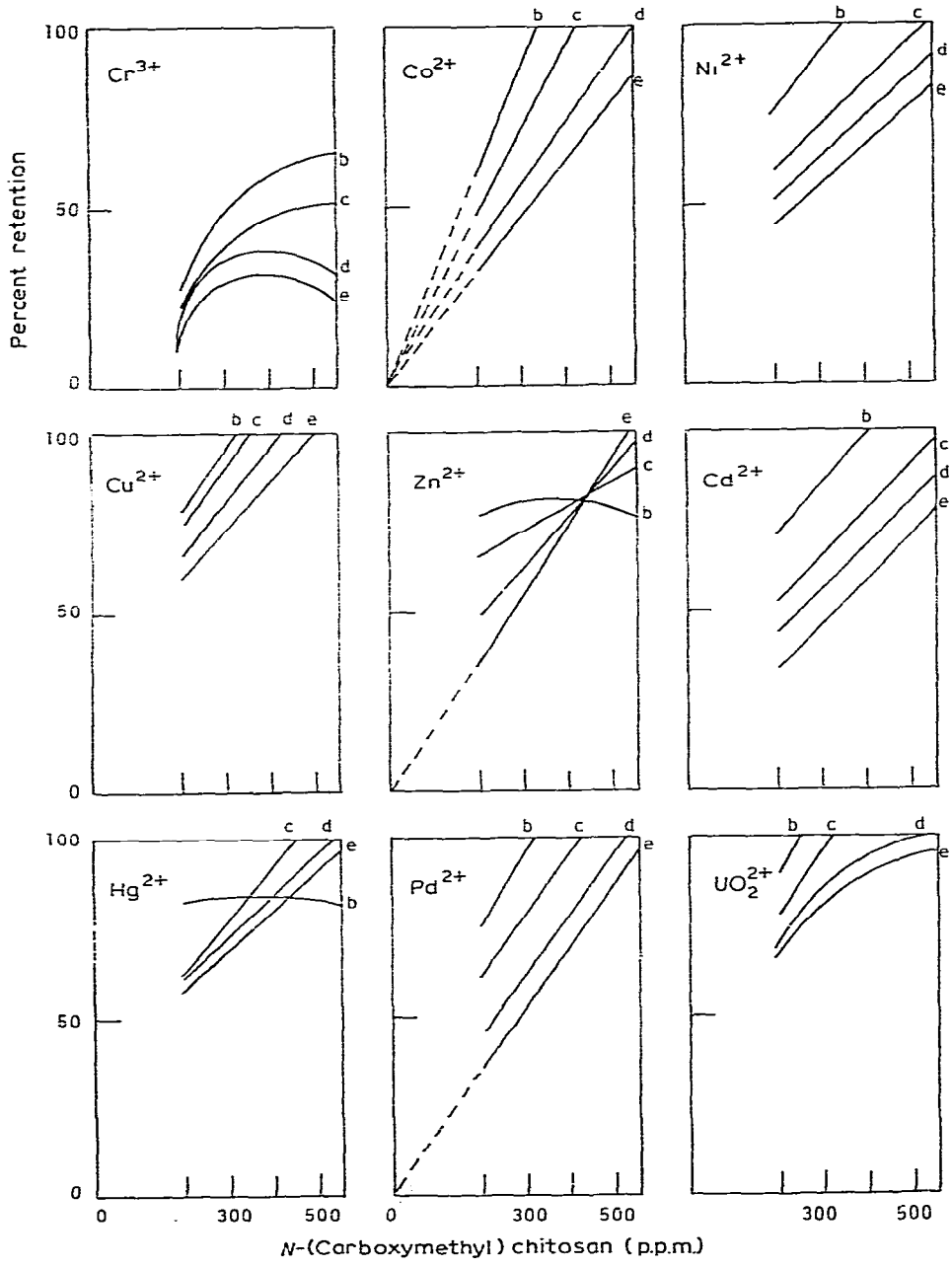


Fig. 10. Percentages of collection for metal ions at various concentrations (a = 0.1, b = 0.2, c = 0.3, d = 0.4, and e = 0.5mM) on *N*-(carboxymethyl)chitosan (fully substituted) *vs* concentration (p.p.m.) of *N*-(carboxymethyl)chitosan.

N-(carboxymethyl)chitosan is 212, one mol of carboxymethylamine residues is associated with 378 daltons (based on the foregoing data and assuming the degree of carboxymethylation to be 56%), and thus, the molar ratios between carboxymethylamine residues and metal ions may be calculated from the data in Fig. 10. These ratios depend upon concentrations, and other groups contribute to the chelation; however, they are found to be close to 3, as expected for the bidentate carboxymethylamino group. Chelation causes cross-linking of *N*-(carboxymethyl)chitosan chains and yields insoluble products.

CONCLUSIONS

Clear solutions of chitosan may be obtained at once by pouring equimolar quantities of chitosan and glyoxylic acid in water. Although solutions thus obtained are acid (pH 3.2–3.5), those solutions whose pH is adjusted at 4.5–6.0 yield gels within 3–4 h, as a consequence of Schiff base and internal-salt formation. The new polyampholyte formed, is soluble in alkaline aqueous solutions if the pH is suddenly raised to ~12, because the Schiff base forms a sodium salt. If the pH is raised too slowly, a significant proportion of the chitosan becomes insoluble.

Gels are also formed at lower pH values if the mixture is warmed; all of them exhibit high capacity for water and methanol. *N*-(Carboxymethylidene)chitosans are soluble, chelating polymers capable of hindering the precipitation of mercury(II) from alkaline solutions.

Mild preparative conditions (aqueous neutral media and room temperature), currently available reagents (glyoxylic acid, chitosan, and reducing agent), and rapidity of the overall preparation (half a working day) permit *N*-(carboxymethyl)-chitosan to be readily obtained. It is a novel polyampholyte, and tailor-made *N*-(carboxymethyl)chitosans may be prepared from a variety of chitosans, differing in molecular sizes, molecular-weight distributions, and degrees of deacetylation, by treating them with various amounts of glyoxylic acid. The conditions of preparation are not such as to degrade the polysaccharides or to alter the degree of acetylation, and the reaction is fast and complete. All of these factors are advantages over the preparation of *O*-(carboxymethyl)chitosans and *O*-(carboxymethyl)chitins.

Whereas some physical similarities exist between *O*-(carboxymethyl)chitosan and *N*-(carboxymethyl)chitosan (moisture content, viscosity fall during the initial two days after dissolution, coagulation by solvents and by neutral salts), the *N*-(carboxymethyl)chitosans are chemically different from *O*-(carboxymethyl)chitosan because they carry the new group $NHCH_2CO_2H$. This function may be chemically compared with glycine: *N*-(carboxymethyl)chitosan may be interpreted as a polysaccharide carrying glycine groups and acetamido groups at C-2, and, from this point of view, could raise interest in fields related to biological chemistry and pharmaceutical chemistry.

The observed functionality of *N*-(carboxymethyl)chitosan may also be related to the well-known chelating agent ethylenediamino(tetraacetic acid) (EDTA): the

sequence N–C–C–O is the same as that which permits formation of pentaatomic rings in metal chelates. It also has the advantage of being a secondary amine (instead of the tertiary amine in EDTA) having the groups interspaced by the hydrophilic glucose ring (instead of the ethylene group in EDTA). With *N*-(carboxymethyl)chitosan, the chelation of metal ions is therefore particularly effective, because of the simultaneous availability of a number of bidentate functions and secondary and tertiary alcoholic groups on a single polymeric chain; this situation leads in fact to the insolubilization of the *N*-(carboxymethyl)chitosan–metal chelates.

The complete solubility of *N*-(carboxymethyl)chitosan at all pH values is another peculiar characteristic of this novel polyampholyte and should favor its applications in various fields.

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