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# Chelating Derivatives of Chitosan Obtained by Reaction with Ascorbic Acid

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*SUMMARY*

*Ascorbic acid immediately dissolves* Euphausia superba *chitosan upon mixing and forms chitosan ascorbate; during the 6-h period after dissolution in water at pH 5–7, ascorbate is oxidized to dehydroascorbate which undergoes Schiff reaction with the amino groups of chitosan, thus yielding a viscous solution of a polymeric ketimine. The latter is characterized by infrared spectrometry, circular dichroism spectropolarimetry, viscometry and alkalimetry. When brought into contact with transition metal ions, the chitosan ascorbate ketimine yields insoluble metal chelates. Upon reduction with sodium cyanoborohydride, the water-insoluble N-[2-(1,2-dihydroxyethyl)tetrahydrofuryl] chitosan (NDTC) is obtained, which shows enhanced capacity for uranium, up to 800 mg U/g from solutions at pH 4-5.*

## INTRODUCTION

Although numerous organic acids have been tested for their ability to dissolve chitosan, ascorbic acid has not so far been investigated (Muzzarelli, 1977; Gross *et al.*, 1982). Ascorbic acid (Counsell & Hornig, 1981; Nobile & Woodhill, 1981) besides being a potentially suitable acid for the formation of a water-soluble chitosan salt (chitosan ascorbate) can also react with chitosan to form chitosan derivatives *via* a Schiff reaction. Mild oxidizing agents such as iodine, Norit and air easily convert ascorbic acid to dehydroascorbic acid, whose carbonyl groups



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are able to react with amines; for example, one of the most widely accepted instrumental methods for the quantitative determination of ascorbic acid involves oxidation followed by a reaction with 1,2-phenylenediamine to produce the fluorophor 3-(1,2-dihydroxyethyl)furo-[3,4-*b*]quinoxaline-1-one, the amount of which is determined by spectrofluorometry according to Deutsch & Weeks (1965) and Keating & Haddad (1982). Since ketimines can be easily reduced, the final product can be tentatively designated as *N*-[2-(1,2-dihydroxyethyl) tetrahydrofuryl] chitosan (NDTC). The presence of the 1,2-dihydroxyethyl groups might give this derivative some properties in common with glycol chitosan, a partially *O*-2-hydroxyethylated chitosan (Muzzarelli, 1977), and hydroxypropyl chitin (Somorin *et al.*, 1982). Hydroxyalkylated polysaccharides are attracting interest in various fields (Mishler, 1982). The presence of monosaccharide units covalently linked to the chitosan chain could lead to enhanced and selective effectiveness in the chelation of transition metal ions (Muzzarelli & Tanfani, 1982; Muzzarelli *et al.*, 1983). We have therefore undertaken the present research to obtain chitosan ascorbate solutions and the ketimine derivatives of chitosan with ascorbic acid.

## EXPERIMENTAL

### Reagents

Chitosan from *Euphausia superba* (Antarctic krill), deacetylation degree 58 ± 4% (Muzzarelli *et al.*, 1981*b*), was supplied by Rybex, Sczesczyn, Poland. Other reagents were supplied by Merck-Schuchardt, West Germany.

### 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*b*).

### Infrared spectrometry

Spectra were recorded using 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.

### Atomic absorption spectrometry

Analyses were carried out according to standard practice using a Perkin-Elmer Model 305 spectrometer equipped with HG-400 graphite atomizer.

### Titration

An Amel pH meter was used in conjunction with Titrisol 0·1 M HCl and NaOH solutions. The chitosan ascorbate samples were 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.

### Metal ion chelation and insolubilization

Solutions of the sulphates of the divalent cations of cobalt, copper, zinc, manganese, cadmium and iron-II as well as uranyl sulphate, chromium-III sulphate and lead-II nitrate were prepared. Aliquots (50 ml) of each solution containing the desired amount of chitosan and ascorbic acid were agitated on a vortex machine at 80 rpm for 1 and 24 h, then filtered through Whatman filter paper and the filtrate analysed by atomic absorption spectrometry (colorimetry, in the case of the uranyl ion, with hydroxy naphthol blue).

### Circular dichroism spectropolarimetry

A Jasco spectropolarimeter Model J-500 was used, with wavelength expansion 10 nm $cm^{-1}$, chart speed 1 cm $min^{-1}$, sensitivity 5 $m^0$ $cm^{-1}$, time constant 4 s, and cell pathlength 1 cm. The polymer concentration was 0·05% in water.

### Preparation of the chitosan ascorbate ketimine

The chitosan powder (0·8 g, 100–200 mesh) suspended in water (100 ml) immediately dissolved upon the addition of ascorbic acid (0·535 g, equimolar to the free amino groups of chitosan). The pH of the resulting solution was between 4 and 5; the solution was limpid, viscous and light yellow. Six hours after preparation this solution was used as a chitosan ascorbate ketimine solution. It was observed that upon further standing the upper part of the solution became darker.

### Preparation of the *N*-[2-(1,2-dihydroxyethyl)tetrahydrofuryl] chitosan (NDTC)

To the chitosan ascorbate ketimine (6 h after preparation) was added sodium cyanoborohydride (0·450 g), and the pH was immediately adjusted to between 6 and 7. The viscous solution was kept under mild stirring overnight. A highly viscous gel resulted which was dialysed against water for three days and then lyophilised.

## RESULTS AND DISCUSSION

### Infrared spectrometry

The infrared spectra recorded on the chitosan ascorbate ketimine showed bands at 1760 and 1710 $cm^{-1}$ due to the $\alpha$–$\beta$ unsaturated cyclic ketones (1620 $cm^{-1}$, mainly due to acetamido groups) together with the other bands commonly found in chitosan spectra. The spectrum obtained on NDTC was similar to that of chitosan itself.

The infrared spectra (Fig. 1) therefore confirm that chitosan reacts with dehydroascorbic acid, because the bands due to the amino groups are altered and the products show the presence of ketone carbonyls. The reduction with sodium cyanoborohydride eliminates the ketimine double bond and yields a product that has a similar infrared spectra to chitosan.

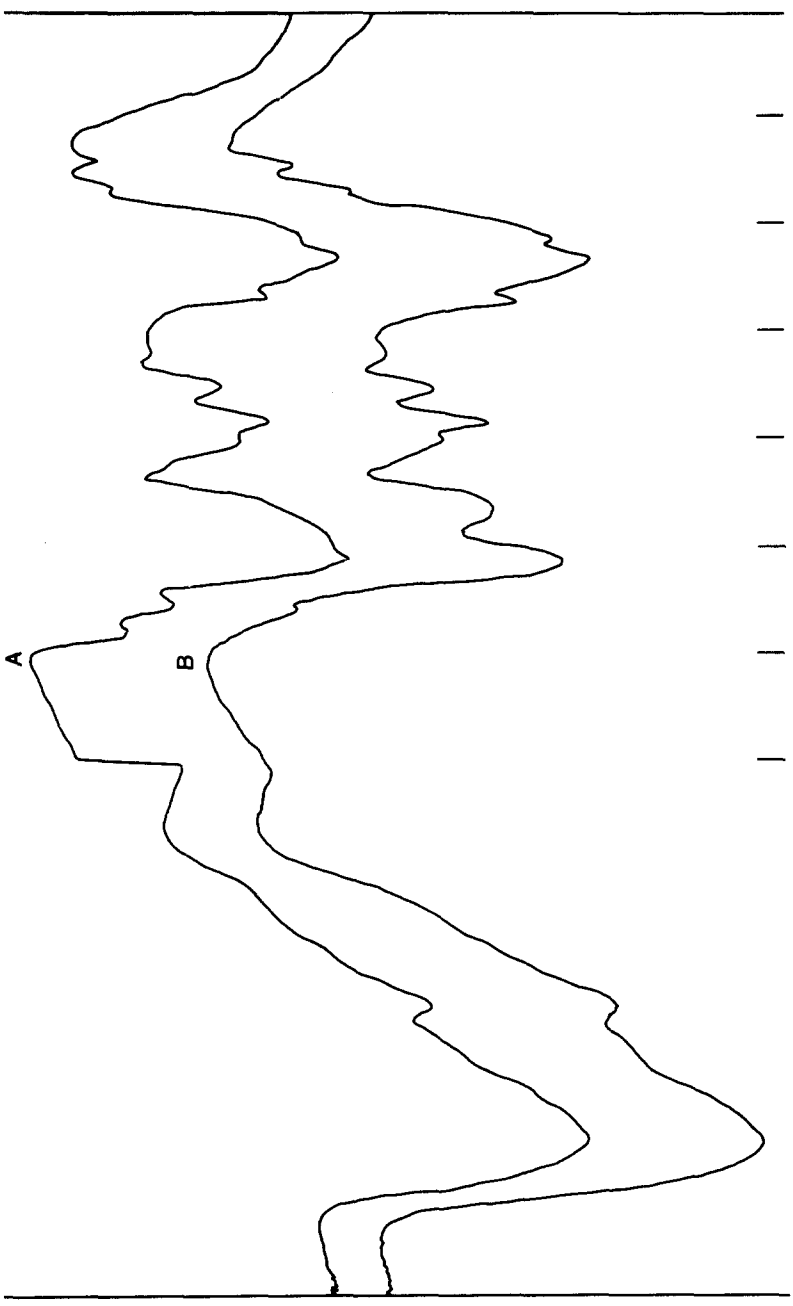


**Fig. 1.** Infrared spectra of derivatives of chitosan and dehydroascorbic acid, in KBr. Curve A: chitosan (300 mg) with ascorbic acid (300 mg) in water (50 ml) after 12 h stirring, 48 h dialysis and freeze-drying. Curve B: as Curve A, after reduction with $NaBH_3CN$ at pH 7·0 for 12 h, 48 h dialysis and freeze-drying.

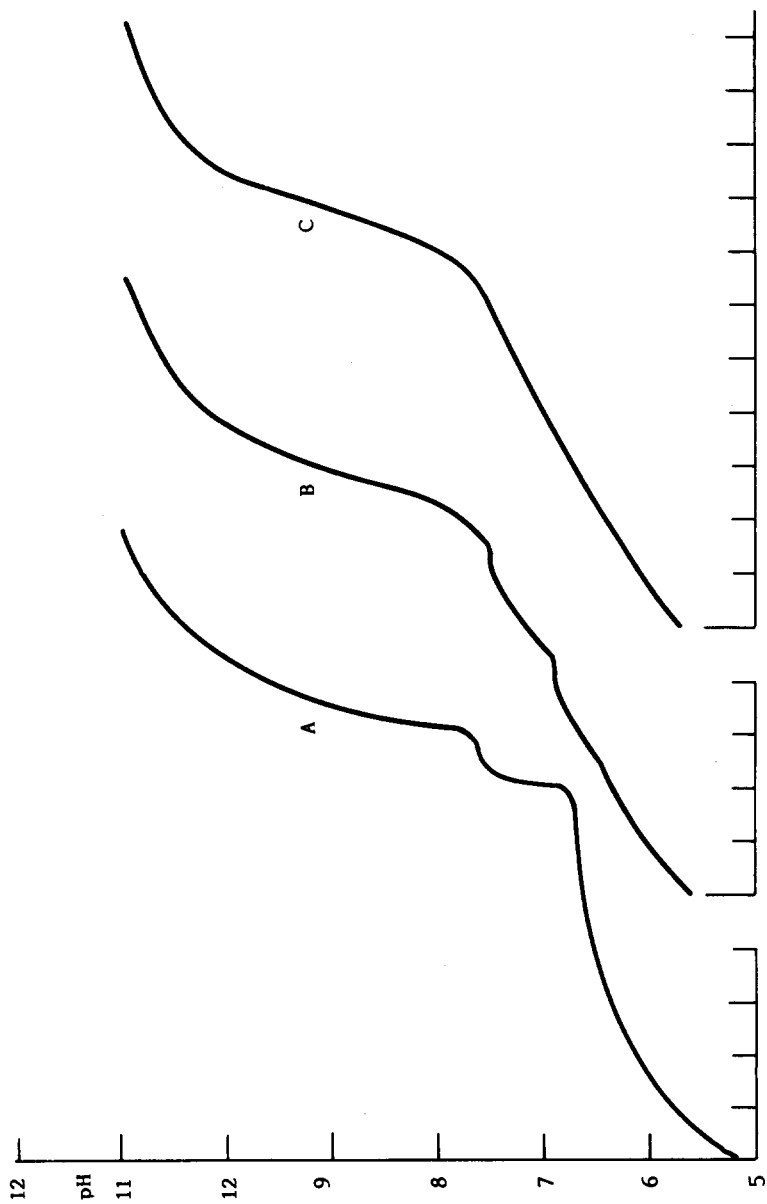


**Fig. 2.** Titration curves for the chitosan ascorbate ketimine obtained under nitrogen. Curve A: just after mixing a chitosan (0·500 g) suspension in water (50 ml) with ascorbic acid (0·318 g) and NaCl (0·327 g). Curve B: 3 h after mixing. Curve C: 6 h after mixing or later. One minute waiting between each addition of 0·1 ml of 0·1 M NaOH.

## Titration

Ascorbic acid alone (0·31687 g, MW 176·14 g $mol^{-1}$) yields a typical symmetric titration curve with an inflection point at 18·0 ml of 0·1 M NaOH solution. The chitosan ascorbate ketimine solution soon after preparation shows a titration curve of the type shown in Fig. 2, curve A. During the next few hours, ascorbic acid reacts with chitosan due to the oxidizing effect of the air dissolved in the stirred solution (Fig. 2, curve B) and therefore the titration curve evolves to that shown in Fig. 2, curve C, which relates to a preparation with an age of 6 h. For 6–24 h after preparation curve C remains unchanged. The original pH of the chitosan ascorbate solution (5·5) is suitable for ketimine formation, even though a slightly higher pH (6·5) gives better yields. This indicates that ascorbic acid and chitosan should be allowed to react for at least 6 h under the conditions described if NDTC has to be prepared.

## Viscometry

The viscosities of the solutions obtained upon dissolving chitosan with ascorbic acid were higher than that of the original chitosan in acetic acid. The viscosity, however, decreased sharply during the few hours after preparation, as shown in Fig. 3 (upper curve). At higher temperatures the viscosities of the chitosan ascorbate ketimine solutions were also depressed (Fig. 3, lower curve). Time and temperature visibly affected the reaction rate and the interactions between modified polymeric molecules were depressed by these two parameters. As shown in Fig. 4, the chitosan ascorbate ketimine is thixotropic at the pH studied. Viscosity is pH dependent; in the acidic range it reached a maximum at pH 4·0, while in the alkaline range the solution had the texture of a soft gel. This trend was quite similar to that followed by *N*-carboxymethyl chitosan (Muzzarelli *et al.*, 1982).

## Circular dichroism spectropolarimetry

The circular dichroism spectra recorded on the ascorbic acid solution alone over a 24-h period showed a negative Cotton band at 194 nm and a positive band at 244 nm; these values did not change with time. The solutions containing both the ascorbic acid and chitosan at pH 6·0

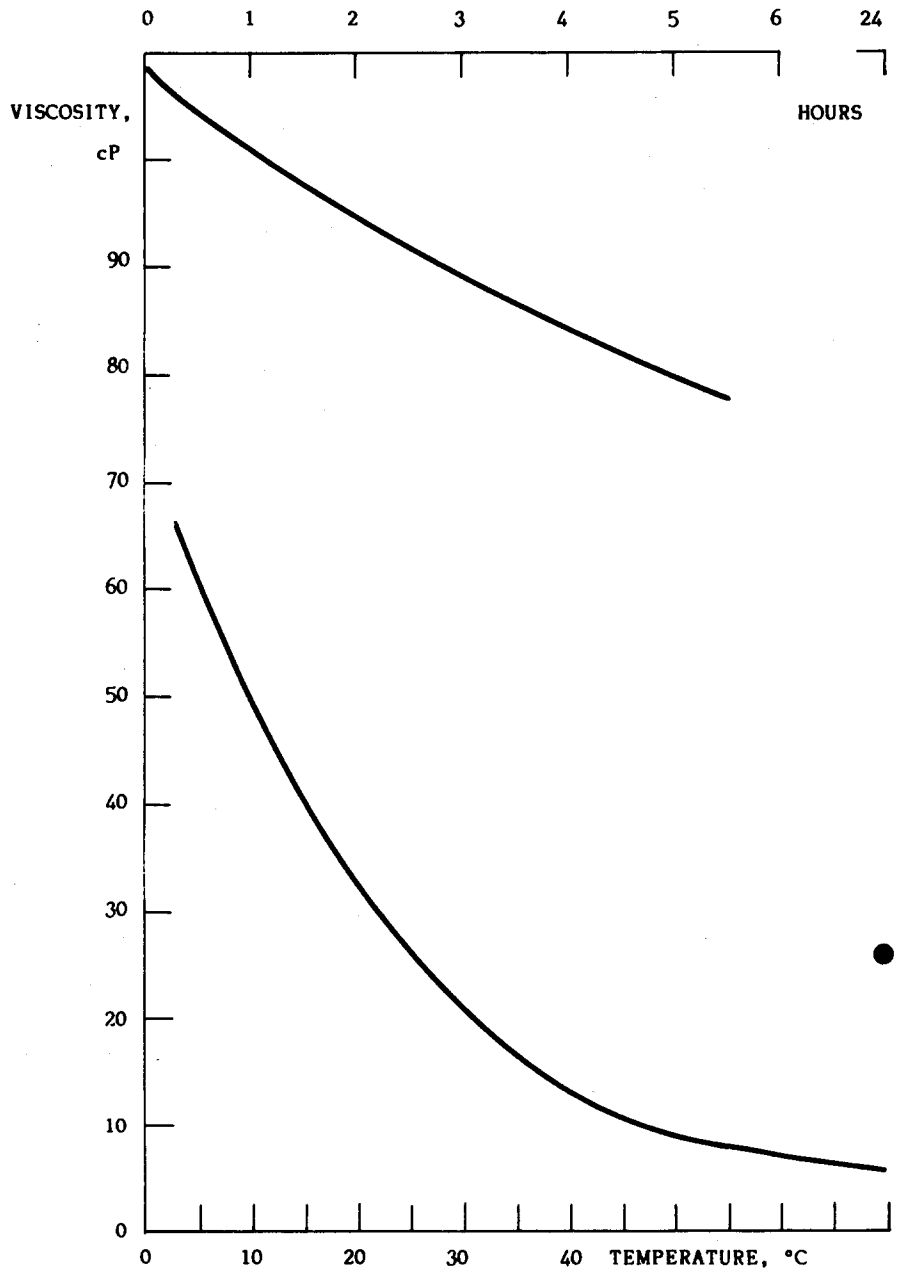


**Fig. 3.** Viscosity of the chitosan ketimine solution as a function of the temperature 1 h after preparation (upper curve) and of the time at 25°C (lower curve). Chitosan, 1%; ascorbic acid, 0·6%. Readings taken at 512 rpm (shear rate = 2770 $s^{-1}$) at the end of the acceleration time. ●, 24 h reading.

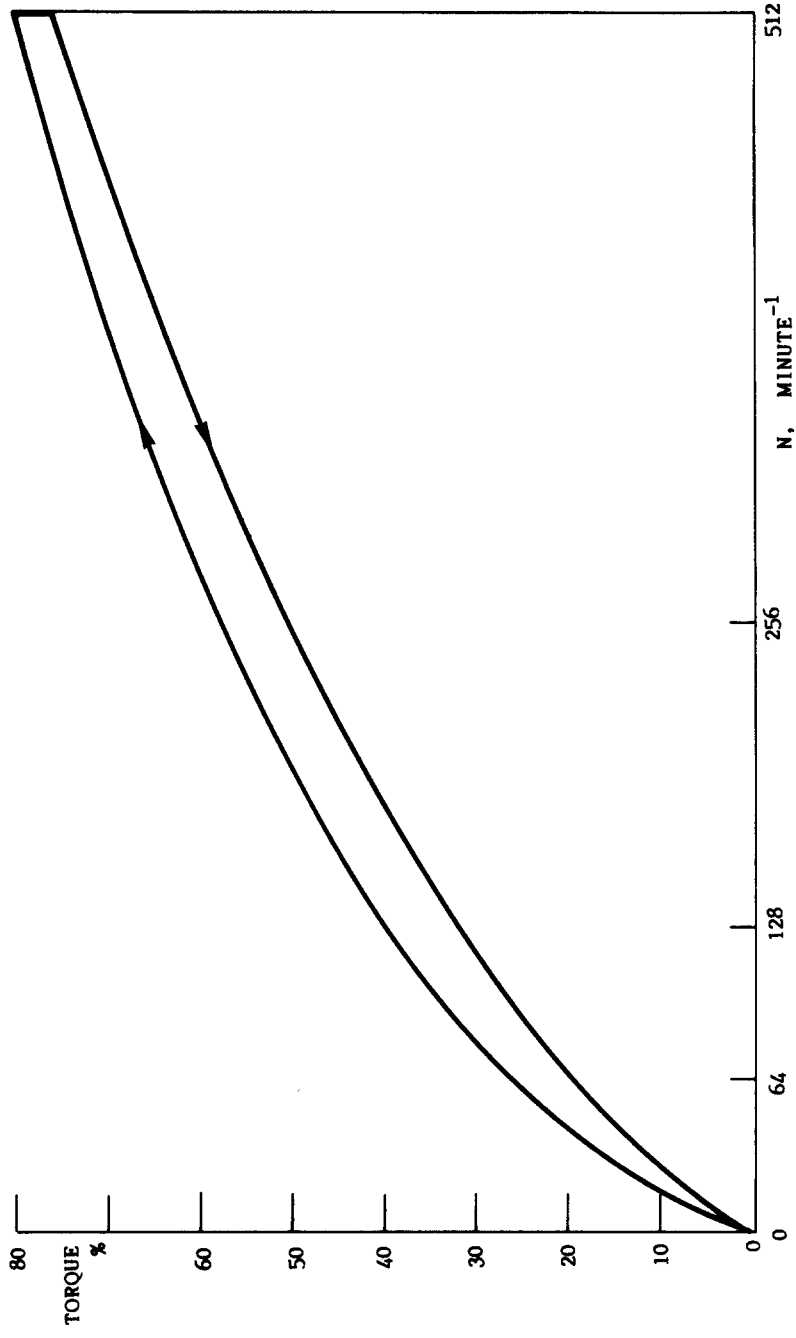


**Fig. 4.** Viscometry on a chitosan (1%) ascorbate (0·6%) ketimine solution at pH 5·5 and 25°C, 15 min after preparation. The splitting of the curves indicates thixotropy. Program: 0·1–2·5–0·1 min. Upper curve, acceleration; lower curve, deceleration. Shear rate $D = 2770\ s^{-1}$ at 512 rpm; shear stress $\tau = 144$ Pa at 512 rpm (at the end of the acceleration time), and $\tau = 137$ Pa after keeping at 512 rpm for 2·5 min (before starting deceleration).

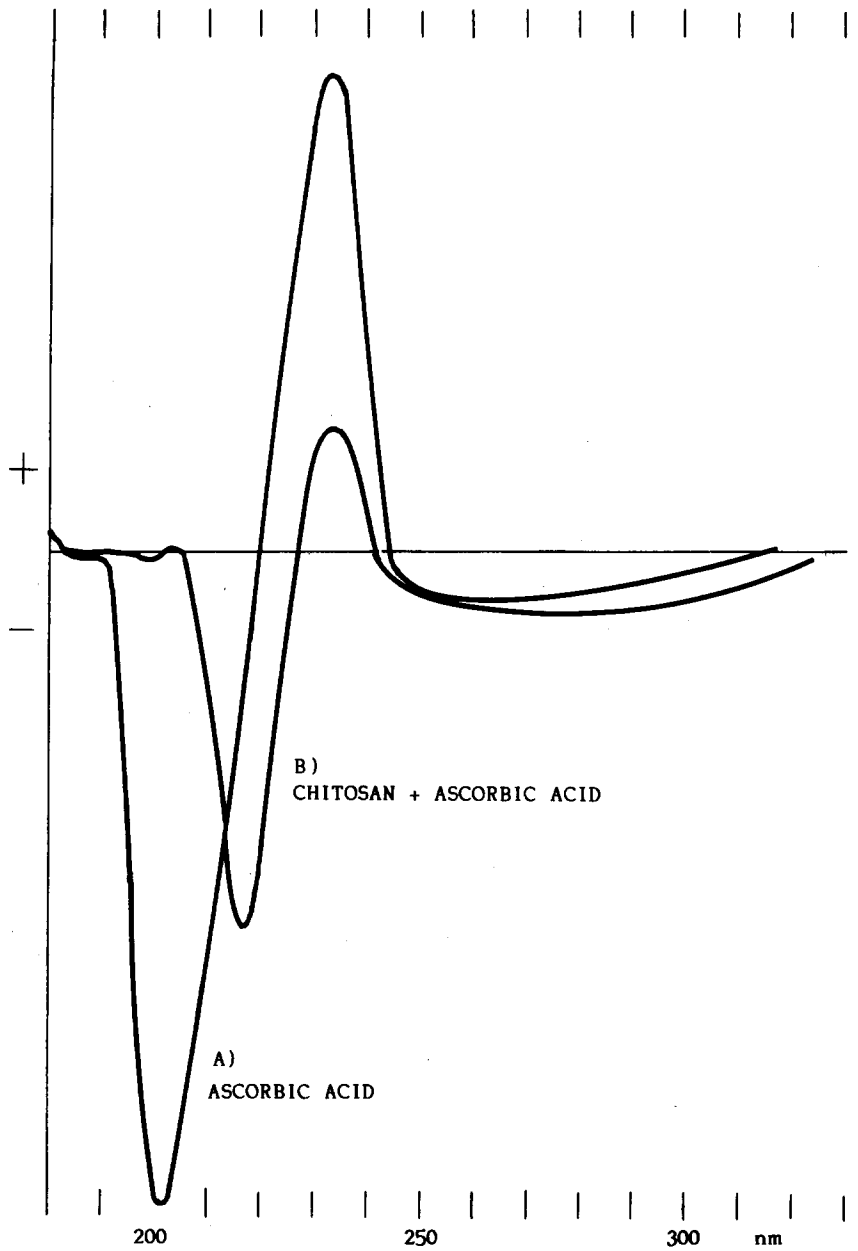


**Fig. 5.** Circular dichroism spectra taken on (A) ascorbic acid solutions and (B) ascorbic acid with chitosan solution, 24 h after preparation. Sensitivity 5 $m^0\ cm^{-1}$; time constant, 4 s.

showed that the negative band was shifted to higher values (200–205 nm) over a 24-h period, while the positive band remained at 244 nm (Fig. 5). Since the latter solutions could contain excess ascorbic acid which would affect the shape of the ketimine spectrum, the solution was submitted to dialysis in order to remove excess ascorbic acid, and after lyophilization, the ketimine was weighed and dissolved in water; the resulting spectrum (Fig. 6) showed a negative band at 213 nm and a

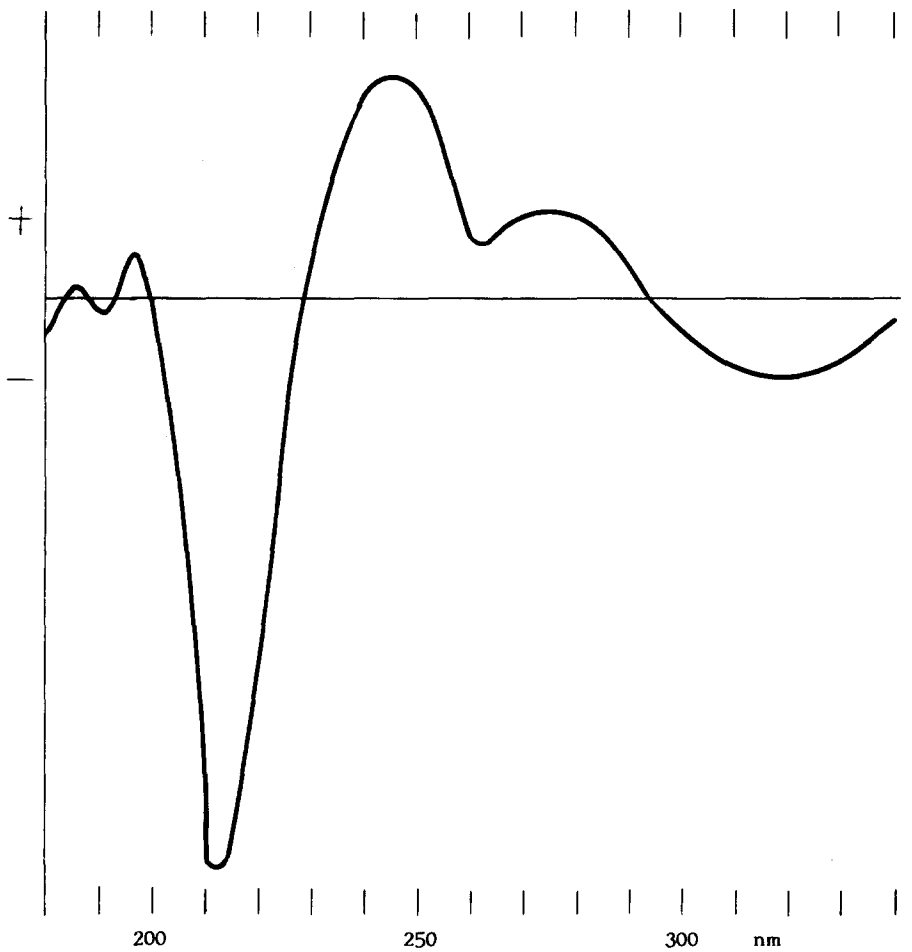


**Fig. 6.** Circular dichroism spectrum taken on the ketimine obtained from ascorbic acid with chitosan after dialysis, lyophilization and dissolution in water (1% w/v). Sensitivity 5 $m^0\ cm^{-1}$; time constant, 4 s.

broader one at 310–320 nm, together with positive bands at 245 and 272 nm.

### Metal ion chelation

When the soluble ketimine obtained from chitosan ascorbate was brought into contact with a transition metal ion solution, the precipitation of a metal ion chelate was observed. For instance, in the case of nickel, a green-brown precipitate was obtained when the polymer (200 mg) was dissolved in a nickel solution (50 ml, 500 mg litre$^{-1}$, pH 8·0); the colour and the consistency depended on concentration and pH.

In the case of iron, a black precipitate was most readily formed when the polymer (200 mg) came into contact with a ferrous ion solution (250 mg litre$^{-1}$, pH 7·7). The copper precipitate obtained under similar conditions was not the cuprous oxide that one would obtain from warm solutions of ascorbic acid, but a voluminous sponge-like precipitate whose colour was red, brown or grey depending on the concentrations used.

In all cases, however, precipitation was not quantitative. Table 1 reports data on batch measurements with 2 mM solutions; particularly high values are obtained for zinc, after 24-h contact, while the other values are much lower than those obtained with other water-soluble derivatives of chitosan.

The batch measurements done on NDTC show the high selectivity of this derivative; while the uptake percentages for most of the metal ions studied are lowered, uranyl ions are very effectively collected within minutes after contact. The most favourable pH value for uranyl ion uptake was around 7; in fact, the uptake values from 5 mM uranium solutions (25 ml) on NDTC (100 mg) were 87% at pH 4·5, 97% at pH 5·5 and 100% at pH values of 6·8, and 7·4, 1 h after contact. After reacting with uranyl ions, the NDTC had a bright yellow–orange colour. In the concentration interval 0·5–5·0 mM the uranyl ion was collected in the batch mode to extents higher than 90%. The capacity for uranium was 800 mg U/g from solutions at pH 4·5.

## CONCLUSIONS

Ascorbic acid is one of the most effective acids for the dissolution of chitosan. Viscous and clear solutions are obtained within minutes after

**TABLE 1**

Collection Percentages of Metal Ions from Aqueous Solutions (50 ml, 2 mM) on Variable Quantities of Chitosan Ascorbate Ketimine Prepared from 0·8% Chitosan Solution and Equimolar Amounts of Ascorbic Acid at pH 7·0

| *Amount of polymer (mg)* | $Cr^{3+}$ | | $Mn^{2+}$ | | $Co^{2+}$ | | $Ni^{2+}$ | | $Cu^{2+}$ | | $Zn^{2+}$ | | $Cd^{2+}$ | | $Pb^{2+}$ | | $UO_2^{2+}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1 h* | *24 h* | *1 h* | *24 h* | *1 h* | *24 h* | *1 h* | *24 h* | *1 h* | *24 h* | *1 h* | *24 h* | *1 h* | *24 h* | *1 h* | *24 h* | *1 h* | *24 h* |
| 50 | 25 | 0 | 37 | 0 | 8 | 8 | 43 | 25 | 75 | 45 | 25 | 89 | 27 | 31 | 8 | 72 | 70 | 72 |
| 100 | 39 | 23 | 30 | 0 | 30 | 8 | 62 | 33 | 79 | 64 | 35 | 94 | 65 | 32 | 82 | 70 | 75 | 75 |
| 150 | 50 | 62 | 30 | 0 | 45 | 34 | 62 | 40 | 81 | 71 | 13 | 95 | 79 | 50 | 83 | 55 | 80 | 83 |
| 200 | 63 | 50 | 40 | 0 | 40 | 28 | 74 | 45 | 72 | 75 | 9 | 88 | 91 | 72 | 80 | 64 | 88 | 91 |

mixing. Under the action of the air in contact with the aqueous solution of chitosan ascorbate, soluble ketimine derivatives of chitosan and dehydroascorbic acid spontaneously form within 6 h at 25°C, or sooner at higher temperatures. They possess characteristic optical properties and have a remarkable ability to form insoluble metal ion chelates.

Upon reduction, the ketimine yields an insoluble white derivative, presumably containing a certain degree of cross-linking due to the plurality of carbonyl groups present on a single dehydroascorbic acid unit. This novel derivative of chitosan exhibits one of the highest capacities for uranyl ion so far reported, this fact being in agreement with the recent observations that *N*-alkyl chitosans (Muzzarelli & Tanfani, 1982; Muzzarelli *et al.*, 1983) and the associations of chitosan and glucans (Hall & Yalpani, 1980; Hall *et al.*, 1981; Muzzarelli *et al.*, 1981*a*; Tsezos & Volesky, 1981, 1982) possess enhanced selectivity and capacity for high atomic number metal ions particularly uranium.

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