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Modification of inulin with amidoxime groups and coordination with copper(II) ions

Dorine L. Verraest[a], Joop A. Peters[a,*], Hennie C. Kuzee[b], Harry W.C. Raaijmakers[b], Herman van Bekkum[a]

[a]*Laboratory of Organic Chemistry and Catalysis, Delft University of Technology, Julianlaan 136, 2628 BL Delft, The Netherlands*
[b]*Coöperatie Cosun U.A., Industrial Inulin Derivatives, Oostelijke Havendijk 15, 4704 RA Roosendaal, The Netherlands*

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Abstract

Inulin modified with amidoxime groups was prepared by reaction of the nitrile groups of *O*-(cyanoethyl)inulin with hydroxylamine. This material has good chelating properties for Cu(II) ions. The coordination of the inulin derivative with Cu(II) has been studied using potentiometry, polarimetry and ^{17}O NMR spectroscopy. At low molar ratio of Cu(II):amidoxime groups ($\rho_L < 0.25$), stable complexes are formed. The optical rotation measurements indicate folding of the backbone to form intramolecular complexes. At higher ρ values, no additional Cu(II) ions are bound by the polymeric ligand. Presumably, no defolding to form 1:1 Cu(II)-amidoxime complexes occurs. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Inulin; Amidoxime; Cu(II)

1. Introduction

Inulin is a linear polymer of β-(2 → 1)-linked fructofuranoside units with one glucopyranose unit at the reducing end (Fig. 1) (Hirst et al., 1950; Suzuki, 1993). It is a reserve polysaccharide found in roots and tubers of plants belonging to the families of the Liliaceae and the Compositae. The most important sources are chicory, Jerusalem artichoke and dahlia, which have an inulin content $> 15\%$ on fresh weight and $> 75\%$ on dry weight. The degree of polymerization (dp) of inulin varies from 2 to about 70 and the average dp depends on the plant origin, the weather conditions during its growth and on its physiological age (de Leenheer, 1996). Values for the average dp of 10–14 for chicory inulin, 20 for dahlia inulin and 6 for inulin extracted from Jerusalem artichoke have been reported (Praznik et al., 1984).

The utilization of inulin has been reviewed by Fuchs (1993) and by de Leenheer (1996). Presently, the main uses of inulin are in the food sector. The major part of the inulin produced is hydrolyzed to yield high fructose syrup, which is mainly used as a sweetener (the sweetness of fructose is 1.5 times that of sucrose on a weight basis).

Inulin is a chemical feedstock from which a variety of chemicals can be produced (Kieboom and van Bekkum, 1985). Hydrolysis to D-fructose and subsequent dehydration leads to hydroxymethylfurfural (HMF) (van Dam et al., 1986), which is considered to be a key chemical (Kieboom and van Bekkum, 1985; van Dam et al., 1986; Fuchs, 1987; Kunz, 1993; Gretz et al., 1993; van Bekkum and Verraest, 1996). Hydrolysis of inulin to D-fructose followed by catalytic hydrogenation yields D-mannitol/D-sorbitol mixtures from which D-mannitol can easily be crystallized (Fuchs, 1987). D-Mannitol is a valuable non-cariogenic, low-calorie sweetener. D-Mannitol can be oxidized, in a partially protected form, at the 3,4-position into two molecules of D-glyceraldehyde or D-glyceric acid, which may serve as chiral building blocks (van Bekkum and Verraest, 1996).

We are engaged in the synthesis of various inulin derivatives and their applications. An obvious way of upgrading inulin is the introduction of carboxylate groups by selective oxidation or by etherification (Fig. 2).

Inulin can be converted to dicarboxy-inulin by direct oxidation with hypochlorite (Besemer and van Bekkum, 1994), or by a two-step process in which first dialdehyde-inulin is prepared using periodate. Subsequent oxidation with chlorite/hydrogen peroxide produces dicarboxy-inulin in a high yield (Besemer et al., 1996). Dicarboxy-inulin

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* Corresponding author

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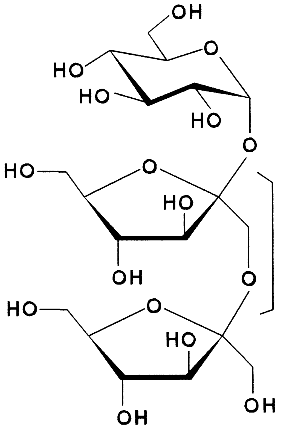

Fig. 1. Inulin ($n = 0$–70).

possesses an excellent calcium sequestering capacity and may be applied as a (co-)builder in detergent formulations.

Highly selective oxidation of the primary alcohol group in inulin can be achieved by the new TEMPO method (de Nooy et al., 1994; 1995). Here TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) is the catalyst and the hypochlorite/bromide combination is the primary oxidant.

Carboxymethylation of inulin is best carried out by operating at low water content using chloroacetate as the reagent (Verraest et al., 1995). *O*-(Carboxymethyl) inulin is showing great promise as a calcium carbonate crystallization inhibitor (Verraest et al., 1996a).

Cyanoethylation of inulin opens a route towards *O*-carboxyethyl inulin (Verraest et al., 1996b). The best synthetic route is a two-step synthesis via the carbamoylethyl derivative (Fig. 3) using hydrogen peroxide at two different alkaline pHs as the hydrolyzing agent. Upon reduction (Li/NH_3/ROH) *O*-aminopropylinulin is obtained (Verraest, 1997). In carboxymethylation and cyanoethylation of inulin the 4-position appears to be the most reactive one (Verraest et al., 1997).

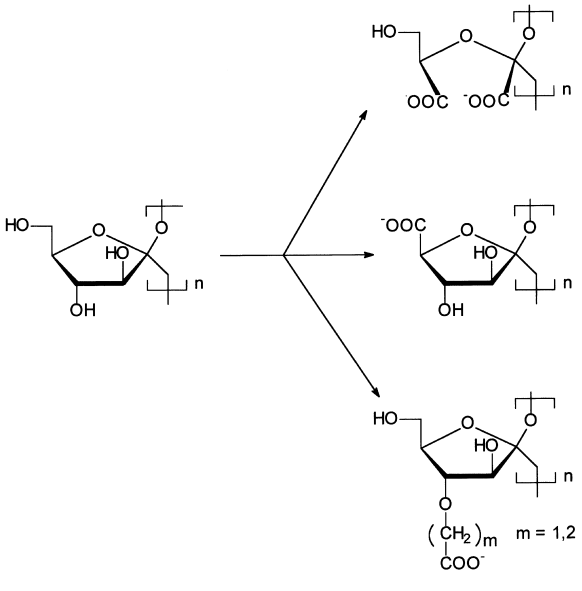

Fig. 2. Inulin based polycarboxylates.

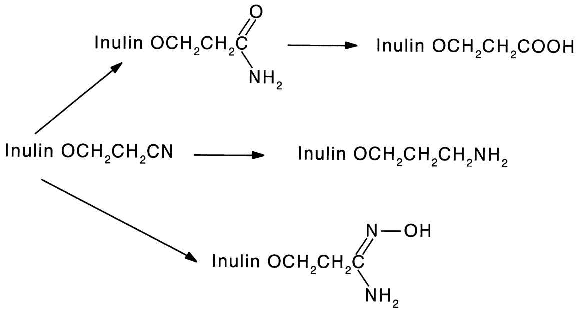

Fig. 3. *O*-Cyanoethyl inulin as precursor of inulin derivatives.

The present paper deals with the preparation and properties of *O*-(3-hydroxyimino-3-aminopropyl) inulin, a new inulin derivative that can be prepared easily from cyanoethylinulin.

2. Experimental

2.1. Materials

Inulin, isolated from chicory root, was a gift from Sensus (Coöperatie Cosun, Roosendaal, The Netherlands) and is commercially available under the name 'Frutafit'. The average dp is 10. Cyanoethylation of inulin was performed as described previously. The complexation studies were carried out with $Cu(NO_3)_2.3H_2O$ (E. Merck, Darmstadt) and $Cu(ClO_4)_2.6H_2O$ (Acros, Geel).

2.2. NMR spectroscopy

^{13}C NMR and ^{1}H spectra were recorded on a Varian INOVA-300 spectrometer and a Varian VXR-400 S spectrometer using D_2O as solvent and *t*-butyl alcohol as internal standard. ^{17}O NMR measurements were performed at 40.697 MHz on a Varian INOVA-300 spectrometer at 348 K using 16-K data points and a spectral width of 20 kHz. The ^{17}O signal of water was shifted by the addition of incremental amounts of $Cu(ClO_4)_2.6H_2O$ to a 0.1 M sample of the ligand (calculation based on amidoximes) in D_2O. A calibration experiment (measurement without ligand) was performed at the same temperature. The ^{17}O shifts were corrected for the shift of the deuterium lock signal due to the presence of paramagnetic metal ions, by monitoring the ^{1}H NMR shift of the internal standard (*t*-butyl alcohol) as a function of the metal ion concentration.

2.3. IR spectroscopy

Infrared spectra were obtained on a Perkin Elmer FT-IR Spectrum 1000 spectrometer. The samples were dispersed in nujol and placed on NaCl discs.

2.4. UV-Vis spectroscopy

UV-Vis spectra were recorded on a Cary 3 Bio photospectrometer.

2.5. Potentiometry

The measurements were carried out using a Unicam IS-Cu Ion selective electrode and a Unicam RE15 double junction reference electrode (Unicam Analytical Systems, Cambridge, UK). Calibration was performed in the range 10^{-2}–10^{-5} M. The ionic strength was maintained at 0.1 M using $NaNO_3$. A Cu(II) concentration reading was taken after each addition of an aliquot of $Cu(NO_3)_2$ solution to a ligand solution (0.1 g in 0.1 l) at pH 6 (0.1 M NaOH). At higher pH, measurement of uncomplexed Cu was impossible due to the formation of Cu(OH) complexes which are not detected by the electrode.

2.6. Optical rotation

The measurements were performed at 25°C on a Perkin-Elmer 241 polarimeter. Optical rotation values of ligand solutions (12.5 mM), containing increasing amounts of Cu(II) were measured.

2.7. Preparation of O-(3-hydroxyimino-3-aminopropyl)inulin

Free hydroxylamine was prepared by neutralization (pH 6.5) of a solution of hydroxylamine hydrochloride (1.8 g, 25 mmol) in 5 ml of water with 2 M NaOH. To this solution, *O*-(cyanoethyl)inulin (1 g, ds 0.95, average dp 10) (Verraest et al., 1996b) was added, and the mixture was heated at 95°C for 5 h. The reaction product was purified using membrane filtration (UTC 60, Toray Industries, Tokyo) at a pressure of 20 bars. After freeze-drying, 0.95 g of *O*-(3-hydroxyimino-3-aminopropyl)inulin (ds 0.76) was obtained. The structure of the reaction product was established using ^{13}C NMR spectroscopy (δ (ppm) 32.0–33.0 ($\mathit{C}H_2C(=NOH)NH_2$), 61.0–87.0 (C1, C3–C6 and OCH_2), 104.5–105.2 (C2), 157.0–158.0 ($C(=NOH)NH_2$). The ds was determined via integration of the ^{1}H NMR spectrum (δ (ppm) 2.75–2.85 ($C\mathit{H}_2C(=NOH)NH_2$), 3.3–4.5 (H1a–b, H3–H5, H6a–b and OCH_2), 5.3–5.5 (H1 of glucose unit). In the IR spectrum of *O*-(3-hydroxyimino-3-aminopropyl) inulin, the characteristic stretching vibration of the nitrile groups at 2240 cm^{-1} had disappeared and a new signal at 1655 cm^{-1} was detected. According to literature, this signal is ascribed to the C=N stretching vibration of the amidoxime groups (Sánchez-Chaves and Arranz, 1996). The N–O stretching is expected at 920 cm^{-1}, but was not detected here due to overlap with the C–O stretching vibrations of the sugar units (900–1300 cm^{-1}). The elemental analysis of a sample of an inulin ether with ds 0.66 of 3-hydroxyimino-3-aminopropyl groups and 0.24 of cyanoethyl groups was in agreement with calculated values for $C_{8.70}H_{16.18}O_{6.41}N_{1.56}$ (taking into account 0.75 mol water per mol monosaccharide unit): found (calc.): C, 41.5 (41.8); H, 6.53 (6.47); O, 41.3 (41.0); N, 8.67 (8.74).

3. Results and discussion

3.1. O-(3-hydroxyimino-3-aminopropyl)inulin

An inulin derivative carrying amidoxime groups was prepared by reaction of *O*-(cyanoethyl)inulin (average dp 10, ds 0.95) (Verraest et al., 1996b; 1997) with an excess of hydroxylamine at pH 6.5 and 95°C. Under the conditions applied, about 80% of the *O*-cyanoethyl groups were converted into *O*-(3-hydroxyimino-3-aminopropyl) groups. Dealkylation was not detected. With this procedure, an inulin derivative with ds 0.76 of *O*-(3-hydroxyimino-3-aminopropyl) substituents and ds 0.19 of *O*-cyanoethyl substituents was, for example, prepared from cyanoethylated inulin with ds 0.95 in 85% yield. The nitrile functions of the *O*-cyanoethyl substituents are assumed not to play an important role during the complexation of the inulin derivative with Cu(II) ions. This was confirmed by the fact that no colored complexes are formed by (O-cyanoethyl)inulin with Cu(II), in contrast with the *O*-3-hydroxyimino-3-aminopropyl derivative.

3.2. Coordination with Cu(II) ions

Amidoximes exist predominantly in the *syn*-hydroxyimino-form which is stabilized by an intramolecular hydrogen bond (Fig. 4) (Hall and Llewellyn, 1956; Millen and Waters, 1968). The compounds behave in water as bases. The pK_a of the conjugated acid of *O*-(3-hydroxyimino-3-aminopropyl)inulin was determined to be 5.0 ± 0.2 using potentiometry. This value is an average of all amidoximes in the polymer and is in agreement with reported values for amidoximes (Millen and Waters, 1968; Ševčík and Grambal, 1975). Amidoximes may coordinate as a non-ionic group, but they generally undergo a metal-assisted hydroxyl proton dissociation upon coordination. The latter type of coordination is expected for Cu(II) ions at pH 6 (the value at which the complexation experiments described below were carried out). The binding is usually bidentate (Fig. 4b) (Ševčík and Grambal, 1975).

Addition of Cu(II) to a solution of *O*-(3-hydroxyimino-3-aminopropyl)inulin gave a green-colored complex. The

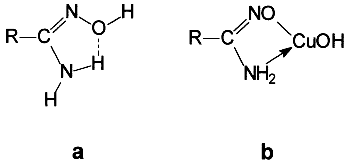

Fig. 4. Structure of amidoxime (a) and coordination with Cu(II) (b).

absorption in the UV-Vis spectrum was found to be pH dependent (UV_{max} 650 nm at pH 4.7; 615 nm at pH 6.0 and 550 at pH 8.0). Apparently, changes in the protonation state of the complex affect the energy levels of the Cu(II) d^9 electrons and consequently the absorption frequencies. When more basic ligands are present, the average absorption maximum of the electronic transitions are known to shift to a lower frequency (Huheey, 1983a), which is in agreement with the results found. At higher pH values (pH > 8), a precipitate of complexes with metal hydroxides was formed.

3.3. Cu(II)-induced ^{17}O water shifts

^{17}O NMR spectroscopy of water is an ideal technique to probe the first coordination sphere of metal complexes in aqueous solution. Chemical shifts and relaxation data may provide information on the bound water molecules and, therefore, indirectly on the binding of other ligands. A series of Cu(II)-induced ^{17}O NMR shift experiments were carried out at 75°C, 40.70 MHz and pH 6. Stepwise addition of a Cu(II) salt to a D_2O solution resulted in a shift of the water ^{17}O signal to a lower frequency as a linear function of ρ_W, the molar ratio of Cu(II) to D_2O (gradient 30 740 ppm). This indicates that the exchange between bound and bulk water is rapid on the NMR time scale. The observed shifts thus represent an average of the chemical shifts of bound and free water molecules. It is known that Cu(II) has a much higher water exchange rate than other transition metals (Lincoln and Merbach, 1995). A value for k_{ex}^{298} of $(4.4 \pm 0.1) \times 10^9\ s^{-1}$ has been reported (Powell et al., 1991). This may be due to the Jahn-Teller effect, which causes a tetragonal distortion of the octahedral aqua-complex. The longer axial bonds facilitate the exchange of the concerning water ligands with bulk water. Furthermore, it has been shown that a rapid interconversion between axial and equatorial waters occurs in the $Cu(H_2O)_6$ complex, which makes the bound water molecules kinetically indistinguishable.

In order to test the applicability of the ^{17}O NMR technique, Cu(II)-induced ^{17}O shifts were measured for ethylenediamine (en) as its coordination to Cu(II) is well-known. Ethylenediamine acts as a bidentate ligand to form 1:1 and 2:1 (ligand/metal) complexes in a square planar configuration, which originates from tetragonal distortion of the octahedral complex due to the Jahn-Teller effect (Huheey, 1983b). The two *trans* water molecules are located at a longer distance from the Cu(II) ion and are only weakly bound. The complexation with a third ethylenediamine molecule is very weak and formation of $Cu(en)_3$ complexes occurs only at extreme high concentrations of en. Values for the stability constants of the complex have been reported to be $\log \beta_1 = 10.5$ and $\log \beta_2 = 20$ (Carlson et al., 1945; Basolo and Murman, 1952; Irving and Williams, 1953).

A plot of the net induced ^{17}O shift of water as a function of ρ_L (ρ_L = mol Cu(II)/mol en) exhibits a change in gradient at $\rho_L = 0.5$ (Fig. 5), corresponding to the change in complex stoichiometry from $Cu(en)_2$ to Cu(en). Here, two water molecules become closely coordinated to Cu(II) and an ^{17}O water shift starts to become apparent. The change in stoichiometry is also reflected by the change in gradient in a plot of the line-width of the ^{17}O signal as a function of ρ_L (Fig. 5).

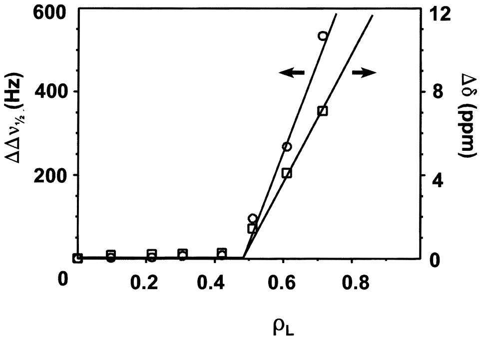

Fig. 5. Cu(II) induced chemical shift (Δδ, □) and line-width ($\Delta\Delta\nu_{1/2}$, ○) of the ^{17}O signal of water as a function of the molar ratio Cu(II):en (ρ_L).

A similar experiment was carried out with *O*-(3-hydroxyimino-3-aminopropyl)inulin (HAI) with ds 0.66 prepared from inulin with an average dp 10. The plot of the net induced ^{17}O shift of water as a function of ρ_L (ρ_L = mol Cu(II)/mol amidoxime functions) and the plot of the line-width of the ^{17}O signal exhibits a change in gradient at $\rho_L \approx 0.25$ (Fig. 6).

Since the exchange between bound and free water molecules is fast on the NMR time scale, the experimental Cu(II)-induced ^{17}O water shift, Δ, is given by Eq. (1),

$$\Delta = q\rho_w\Delta_c \tag{1}$$

where Δ_c is the shift of bound water molecules in the Cu(II) complex, q is the number of water ligands in the first coordination sphere of Cu(II) and ρ_w is the molar ratio Cu(II)/D_2O. It may be assumed that Δ_c is almost completely of contact origin.

For $Cu(en)_2$, the gradient of the Cu(II)-induced ^{17}O shift versus ρ_w was essentially zero. Apparently, the coordination of the two *trans* water molecules in the $Cu(en)_2$ complex is

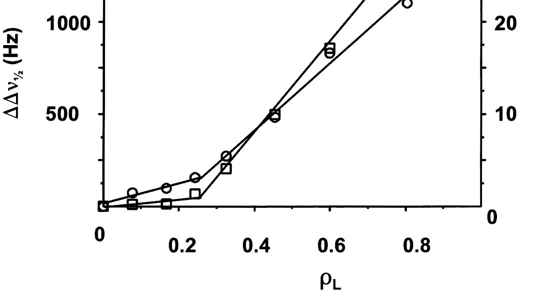

Fig. 6. Cu(II) induced chemical shift (Δδ, □) and line-width ($\Delta\Delta\nu_{1/2}$, ○) of the ^{17}O signal of water as a function of the molar ratio Cu(II):amidoxime of the ligand *O*-(3-hydroximino-3-aminopropyl)inulin.

so weak, due to the tetragonal distortion ascribed to the Jahn-Teller effect, that their ^{17}O signals do not undergo a Cu(II)-induced shift. Consequently, the Cu(II)-induced ^{17}O shift of the water molecules in the absence of ligand may probably be ascribed exclusively to the four equatorial water molecules of the octahedral $Cu(H_2O)_6$ complex.

At $\rho > 0.5$, the slope was found to be 20 692 ppm. This shift can probably be ascribed to two equatorial water molecules in the Cu(en) complex. For *O*-(3-hydroximino-3-aminopropyl)inulin (HAI), the plot of the Cu(II)-induced ^{17}O shift versus ρ_L, showed again a zero gradient at low ρ_L and a gradient of 27 846 ppm at $\rho_L > 0.25$. This indicates that at $\rho_L < 0.25$, complexes are formed with at least four coordination sites (in analogy with $Cu(en)_2$). The slope at $\rho_L > 0.25$ is of the same magnitude as the slope found in the absence of ligand. Probably, no additional Cu(II) ions are bound by HAI at $\rho_L > 0.25$.

The results of the Cu(II)-induced ^{17}O water shifts are tentatively interpreted in the way that one Cu(II) ion is coordinated by two bidentate amidoxime ligands of HAI at $\rho_L < 0.25$ to form such 2:1 complexes, the backbone of HAI has to fold to bring two amidoxime substituents close to a Cu(II) ion. The formation of intermolecular Cu(II) complexes is not expected, as the viscosity of the ligand solution is not increased significantly by addition of Cu(II) ions. The unexpected change in gradient at $\rho_L \approx 0.25$, rather than at $\rho_L = 0.5$, may be explained by sterical hindrance preventing about half of the amidoxime groups to form stable 2:1 complexes or by coordination of the remaining amidoxime groups at the axial positions of the 2:1 complexes. Upon an increase of ρ_L ($\rho_L > 0.25$), essentially no additional Cu(II) ions are bound by HAI, showing that these complexes are much more stable than 1:1 complexes. Apparently, defolding of the inulin backbone in order to form 1:1 Cu(II)-amidoxime complexes does not occur. This interpretation implies that for *O*-(3-hydroximino-3-aminopropyl)inulin with an average dp 10 and ds 0.66, on the average one to two Cu(II) ions are bound per molecule (Fig. 7).

The conformational changes of a ligand during complexation of metal ions may be monitored by optical rotation measurements. Fig. 8 shows the change in optical rotation of the HAI–Cu(II) complex with respect to that of the free ligand, $\Delta[\alpha]_D^{25}$, as a function of ρ_L. The optical rotation decreases sharply when Cu(II) ions are added to the ligand solution. This change may be ascribed to coordination of Cu(II) ions. Between $\rho_L = 0.1$ and $\rho_L = 0.4$, an increase in optical rotation values is observed, indicating that the addition of Cu(II) ions brings about a drastic conformational change in the ligand. Probably, intensive folding of the inulin backbone takes place in order to form intramolecular 2:1 complexes, as described above. When $\rho_L > 0.4$, the $[\alpha]_D^{25}$ value is constant. Apparently, the conformation of the formed HAI–Cu(II) complexes is not changed upon further addition of Cu(II), which confirms the results obtained by the measurements of the Cu(II)-induced ^{17}O shifts.

3.4. Potentiometric study of the complexation with Cu(II)

In order to determine stability constants of the Cu(II) complexes, a solution of 100 mg of *O*-(3-hydroxyimino-3-

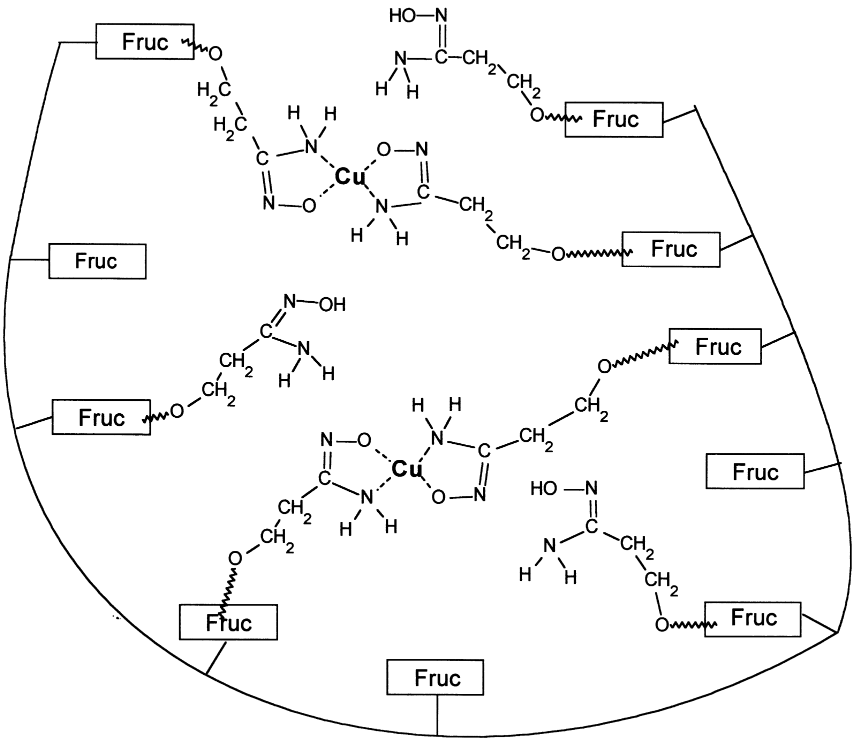

Fig. 7. Schematic representation of the proposed structure of a Cu(II) complex of an inulin molecule with amidoxime substituents (dp 10, ds 0.7).

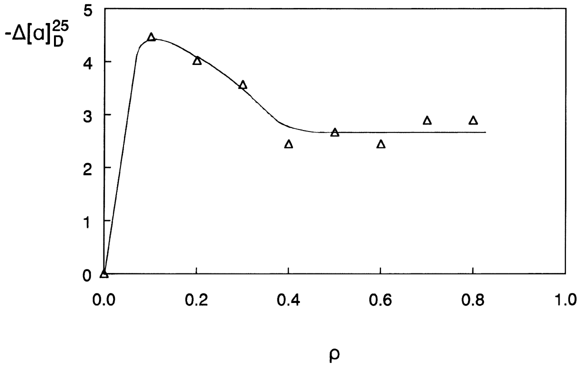

Fig. 8. Optical rotation of HAI as a function of the molar ratio Cu(II):amidoxime groups. The curve is a guide to the eye.

aminopropyl)inulin in 100 ml 0.1 M $NaNO_3$, was titrated with a Cu(II) solution (0.1 M), the course of the complexation being followed by measuring the concentration of uncomplexed Cu(II) present using a Cu(II) ion-selective electrode. All measurements were performed at pH 6. In Fig. 9, an isotherm of the binding of Cu(II) ions to HAI is plotted. It is clear that up to $\rho_L = 0.18$, essentially all Cu(II) added is bound to HAI. At higher ρ_L values ($\rho_L > 0.40$), the amount of additional Cu(II) ions bound by the ligand is low, confirming the results described above. The stability constant of the complex ($\log K$) was determined from the titration measurements. Calculation of the complex concentration was based on a complex stoichiometry of 2:1, as suggested by the Cu(II)-induced ^{17}O shift measurements. The $\log K$ value was found to decrease as a function of ρ_L, pointing to anti-cooperative binding. The intrinsic stability constant, defined as the value for the first Cu(II) ion binding and determined by extrapolation of the curve to $\rho = 0$, was found to be $\log K_{intr.} \approx 8.5$. For the model compound 3-ethoxy-propionamidoxime, the calculation of the $\log K$ value based on a 1:1 stoichiometry was found to be independent of ρ_L ($\log K = 2.9 \pm 0.2$). The large difference in $\log K$ values between the monomeric and polymeric ligands may be explained by the difference in complex stoichiometry and by the fact that complexation of metal ions by different binding sites in one molecule is energetically more favorable than by monomeric binding sites.

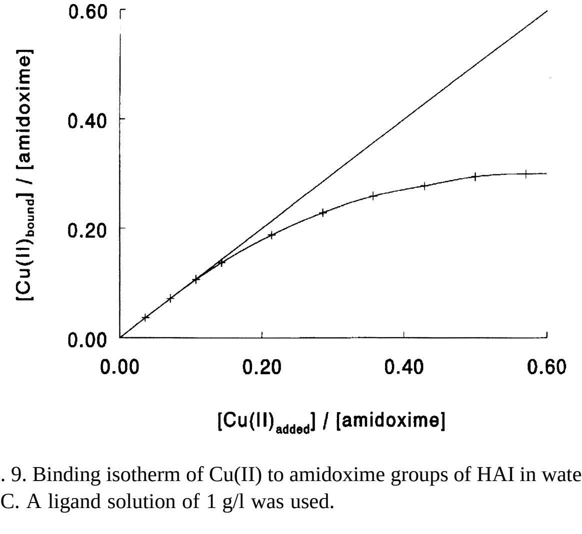

Fig. 9. Binding isotherm of Cu(II) to amidoxime groups of HAI in water at 22°C. A ligand solution of 1 g/l was used.

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