Research, Port Sunlight, England, for helpful discussions.

Registry No. Sodium myristate, 822-12-8; amylose, 9005-82-7.

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Complex Formation between Copper(II) and Poly(*N*-methacryloyl-L-asparagine)

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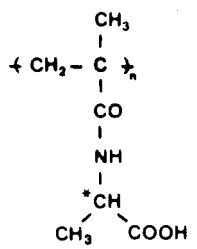
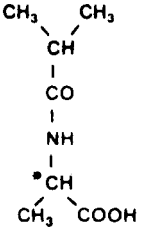
ABSTRACT: The complexes formed between copper(II) and a polymeric ligand derived from L-asparagine, poly(*N*-methacryloyl-L-asparagine) (PNMAsn), have been investigated by potentiometric titration, electronic spectroscopy, and circular dichroism. *N*-Isobutyroyl-L-asparagine (NIBAsn) was also synthesized and studied for comparison with its polymeric analogue. It was found that NIBAsn forms only one weak complex with copper(II), with a bonding between the metal and the carboxyl group. With PNMAsn, three complexes have been demonstrated. Some of them involve the deprotonation of the amide group. The difference between the polymeric ligand and the model molecule is discussed in terms of the high local concentration of the ligand and electrostatic interactions.

Introduction

In previous papers[1,2] we have reported the study of copper(II) complexes of an optically active polyacid derived from alanine, poly(*N*-methacryloyl-L-alanine) (PNMA) (I).



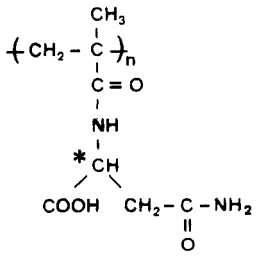
PNMA (I)

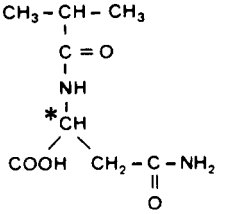


NIBA (II)

The complexing properties of the model molecule of PNMA, *N*-isobutyroyl-L-alanine (NIBA) (II), were also investigated in order to demonstrate the role of the macromolecular chain in the process of complex formation. It was shown that NIBA forms only a weak 1:1 ligand:metal complex through the carboxyl group.

In contrast, PNMA forms a series of different complexes depending on the pH. Some of these complexes involve the deprotonation of the amide group with formation of 1:1 species in one side chain or 2:1 species between two side chains of the polymer (two metal–nitrogen bonds). The present paper reports results obtained in the study of another polyacid derived from an amino acid, poly(*N*-methacryloyl-L-asparagine), with a side chain containing a carboxyl group, a secondary amide, and a primary amide (PNMAsn (III)). PNMAsn has three potential binding sites, and three chelates of different sizes may be formed.





The complexing properties of PNMAsn and its model molecule, *N*-isobutyroyl-L-asparagine (NIBAsn) (IV), have been investigated by potentiometric titration, electronic spectroscopy, and circular dichroism.

Experimental Section

Samples. *N*-Methacryloyl-L-asparagine was prepared from methacryloyl chloride (Fluka) and L-asparagine (Merck) according

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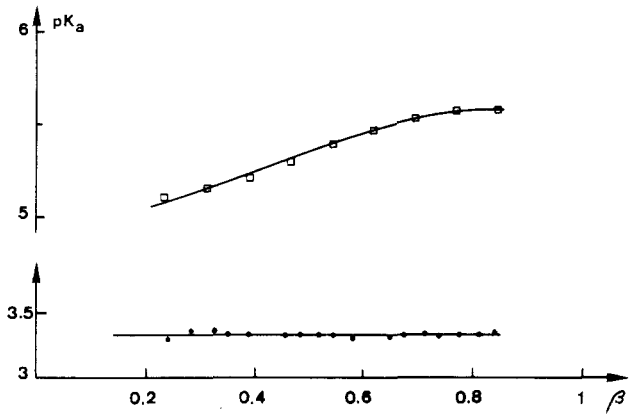


Figure 1. Variation of the apparent pK, pK_a, of PNMAsn (□) and NIBAsn (●) as a function of the degree of dissociation β in 0.1 M $NaClO_4$.

to the method of Kulkarni and Morawetz[3] ($[\alpha]^{20}_{546}$ −24.4° in water). The model molecule, NIBAsn (IV), was obtained from isobutyroyl chloride and L-asparagine ($[\alpha]^{20}_{546}$ −36° in water; mp 152 °C). The monomer and model molecule were purified by recrystallization from ethyl acetate. The test with ninhydrin revealed that the samples contained no trace of residual amino acid. They were also characterized by IR and NMR spectroscopy.

PNMAsn (II) was obtained by free radical polymerization of *N*-methacryloyl-L-asparagine in dioxane, initiated by AIBN at 60 °C ($[\alpha]^{20}_{546}$ −24.4° in water). PNMAsn was purified by extensive dialysis against methanol and water and then recovered by freeze-drying. It was also characterized by IR and NMR spectroscopy. NIBAsn and PNMAsn were also acid–base titrated and were found to contain significant amounts of water (2–5 wt %) even after freeze-drying. Thus the samples were kept in a desiccator, and the exact concentrations of the stock solutions were determined by potentiometry. In the potentiometric study of the complexation, the concentration of the ligand was also refined by calculation.

Copper perchlorate (Merck) was used as the metal ion source.

Methods. Absorption spectra were recorded at room temperature with a Cary 118 spectrophotometer. The molar extinction coefficient ϵ, expressed in $L \cdot mol^{-1} \cdot cm^{-1}$, refers to copper for the visible range (d–d transitions of the metal) and to the ligand for the UV range (intraligand and charge-transfer transitions) CD spectra were recorded at room temperature with a Jobin-Yvon Mark III dichrograph flushed with dry nitrogen. As for ϵ, the dichroic signal, $\Delta\epsilon$, refers to copper or ligand, depending on the wavelength range. Potentiometric titrations were made with a fully automated titration apparatus controlled by a Hewlett-Packard HP 9825 calculator and composed of a Radiometer pHM 64 pH meter, a Schott N65 combined electrode, and a Gilmont microsyringe for addition of the titrant. All titrations were carried out at 25 °C under nitrogen. Experimental data were recorded on a magnetic tape cartridge for further processing with computer programs.

Solutions with molar ratio R ([ligand]/[metal]) ranging from 1 to 8 were used throughout this study, but as results were very similar, only data obtained for R = 8 are reported in this paper.

Results and Discussion

Potentiometric Titrations. Potentiometric titrations of NIBAsn and PNMAsn were carried out in pure water or in 0.1 M $NaClO_4$.

Figure 1 shows typical modified titration curves in 0.1 M $NaClO_4$ where the apparent pK, pK_a, is plotted vs. the degree of dissociation β, according to

$$pK_a = pH - \log\left[\frac{\beta}{1-\beta}\right] = pK_0 + B\psi \qquad (1)$$

where pK_0 is the intrinsic dissociation constant and $B\psi$ is an electrostatic term. $B\psi$ depends on repulsions between the charged carboxylate groups and increases with increasing β.[4] For NIBAsn, pK_a is nearly constant, which is usual for a simple molecule, and equal to 3.40. The pK_0 of NIBAsn was also calculated from the titration curve by using the computer program MUPROT[5] for the determination of protonation constants, and the value 3.35 was obtained. For PNMAsn, pK_a increases smoothly, reflecting a progressive expansion of the macromolecule as the ionization increases. No conformational change is observed.

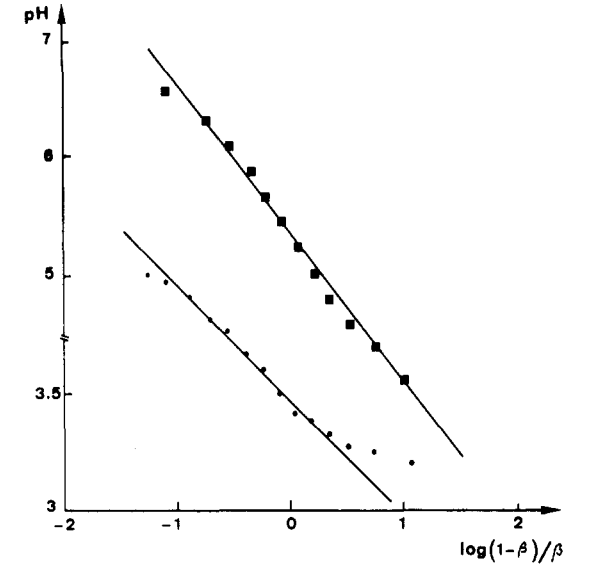


Figure 2. Henderson–Hasselbach plot for PNMAsn (■) and NIBAsn (●) in 0.1. M $NaClO_4$.

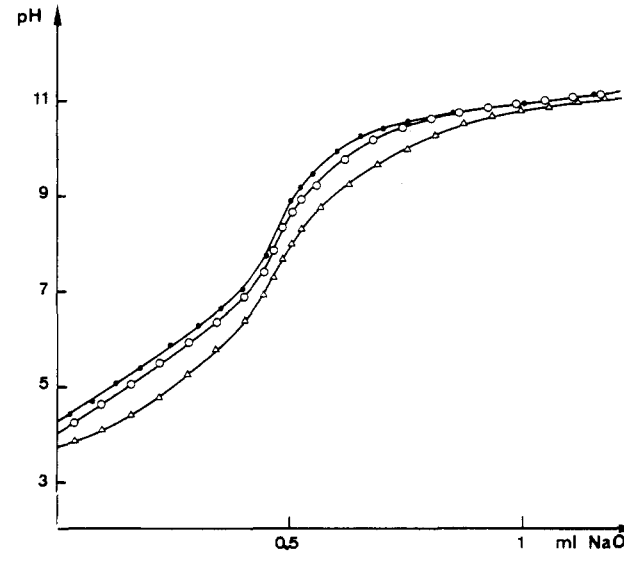


Figure 3. Titration curves of PNMAsn (●) in pure water, no copper; (○) in 0.1 M $NaClO_4$, no copper; and (△) in 0.1 M $NaClO_4$, [PNMAsn]/[Cu] = 5.

Henderson–Hasselbach curves corresponding to the equation

$$pH = pK_{1/2} - n \log [(1-\beta)/\beta] \qquad (2)$$

are plotted in Figure 2. For NIBAsn in 0.1 M $NaClO_4$, $pK_{1/2}$ = 3.4 and n is close to unity as expected (n = 1.02). For PNMAsn, $pK_{1/2}$ and n decrease from 5.96 to 5.35 and from 1.62 to 1.25, respectively, when changing from pure water to 0.1 M $NaClO_4$. The added salt reduces the electrostatic interactions but they remain important.

Figure 3 shows the effect of added copper (R = 5) on the titration curve of PNMAsn. The decrease of the pH

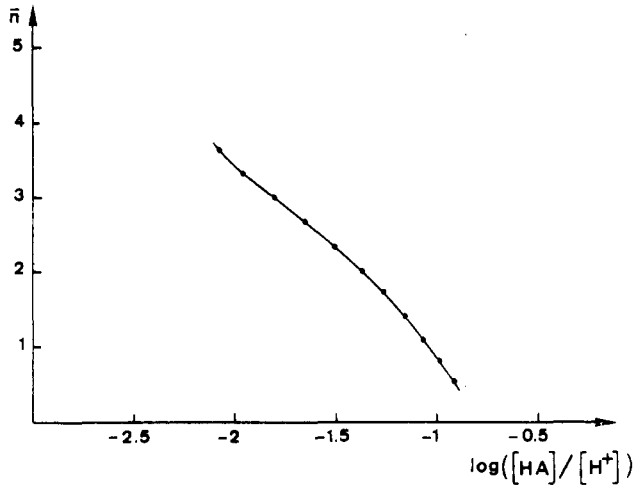


Figure 4. Formation curve for the PNMAsn–Cu complexes (R = 5) in 0.1 M $NaClO_4$.

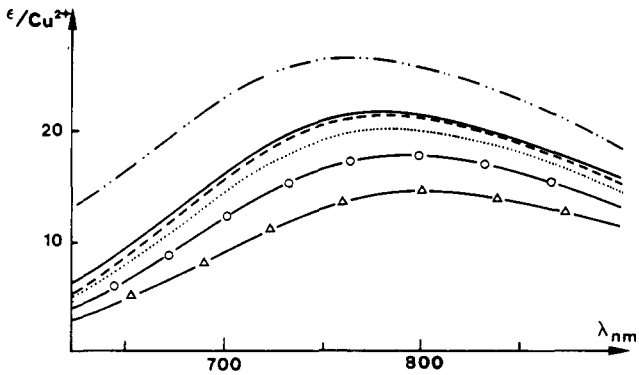


Figure 5. Visible absorption spectra of NIBAsn:Cu, R = 8, as a function of pH: (△) pH 2.55; (○) pH 3.20; (···) pH 4.65; (---) pH 5.0; (—) pH 5.7; (-··) pH 6.25.

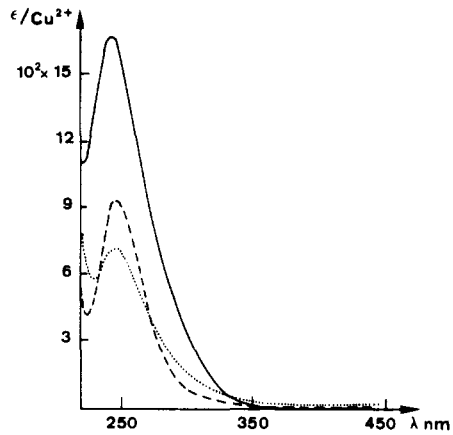


Figure 6. Differential UV absorption spectra of NIBAsn:Cu, R = 8, as a function of pH (some mixture pH 2.1 in the reference cell): (···) pH 3.85; (---) pH 4.9; (—) pH 6.05.

indicates that the ionization of the carboxyl groups is perturbed by their complexation with the metal ion. The data of the potentiometric titration curves have been treated according to the method of Bjerrum,[6] as modified by Gregor et al.[7] and Mandel and Leyte[8] for polyacids. A typical formation curve is given in Figure 4, where $\bar{n}$, the average number of ligands bound to one metal ion, is plotted vs. p([HA]/[H^+]) ([HA] is the molar concentration of un-ionized ligand). If we assume that the complex formed between PNMAsn and copper should be a 2:1 complex involving two carboxylate groups, the $\bar{n}$ value should be 2 at high pH (low p([HA]/[H^+]) values). On the contrary $\bar{n}$ further increases, suggesting that other functional groups, such as the amide groups are involved in the complex. n_{H^+}, the number of protons titrated per metal ion has been calculated from the experimental data. Near pH 7, $n_{H^+} = R + 1$, and at pH ~9, $n_{H^+} = R + 2$. This means that one or two additional protons are liberated by the formation of the complexes, coming from the amide group. This is a first indication for the deprotonation of the amide group in the presence of copper.

From the value of ([HA]/[H^+]) at $\bar{n}$ = 1, the constant B_2 for the equilibrium

$$2RCOOH + Cu^{2+} \rightleftharpoons (RCOO)_2Cu + 2H^+ \quad (3)$$

may be calculated in the usual way.[7,8]

Then the constant K_2 for the equilibrium

$$2RCOO^- + Cu^{2+} \rightleftharpoons (RCOO)_2Cu \quad (4)$$

is obtained by using the relation

$$B_2 = K_2 K_{1/2}{}^2 \quad (5)$$

where $K_{1/2}$ is the value determined from the Henderson–Hasselbach plot.[7]

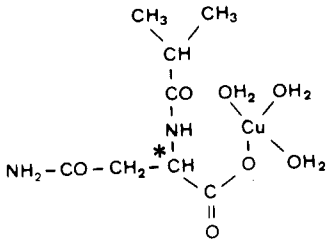
For PNMAsn in 0.1 M $NaClO_4$, $B_2 = (1 \pm 0.05) \times 10^{-2}$ and $K_2 = (5 \pm 0.3) \times 10^8$ are found (average of seven measurements at different [ligand]/[metal] ratios). These are very close to values reported for poly(acrylic acid),[7] poly(methacrylic acid),[8] and poly(glutamic acid)[9] and confirm that the first complex formed in the PNMAsn–Cu system is a very stable chelate of the $(RCOO)_2Cu$ type.

On the other hand, NIBAsn gives with copper a very weak 1:1 complex with a bonding between copper and one carboxyl group. This has been determined from the analysis of the titration curves using the MUCOMP computer program.[10]. The stability constant K_1 of the NIBAsn:Cu complex is about 10. At pH >6 precipitation of copper hydroxide is observed with NIBAsn, whereas PNMAsn:Cu solutions remain clear, indicating that all copper has been complexed before this pH. This has been confirmed by measurements with a copper ion selective electrode.

The difference between NIBAsn and PNMAsn is due to the polymeric nature of the latter, for which the vicinity of two side chains with carboxyl groups allows the formation of a chelate. Another important effect is the high electrostatic field of the polyelectrolyte, which exerts a strong attraction on the metal ion.

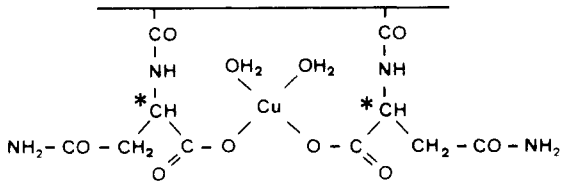
Spectroscopic Study. In the visible range (Figure 5) the absorption spectra of NIBAsn:Cu indicate a slight blue shift of the d–d transitions of Cu(II) (800 to 750 nm) when the pH is increased. The wavelength shift and the increase of ϵ indicate the replacement of a water molecule bound to copper by one carboxylate group.[11] In the UV range (Figure 6), the differential absorption spectra, recorded with a NIBAsn:Cu mixture at pH 2.1 in the reference cell, show the presence of an intense absorption band near 250 nm, increasing with pH. This is attributed to a charge-transfer transition $COO^- \rightarrow Cu$.[12-14]

No dichroic signal is observed for the NIBAsn:Cu system in the visible range, whatever the pH is. This means that the asymmetric carbon is not involved in a chelate ring. In the UV range (Figure 7), the dichroic spectra show a negative band around 250 nm, which contains at least two contributions, i.e., the $n \rightarrow \pi^*$ amide transition[15,16] and the $COO^- \rightarrow Cu$ charge-transfer transition.[12-14] Thus, results of the spectroscopic study confirm the formation of the following complex in the NIBAsn:Cu system:



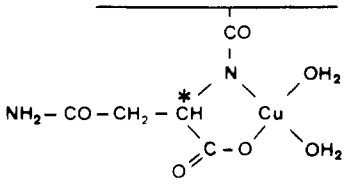
Results obtained with PNMAsn are very different. The visible absorption spectra show a continuous blue shift of the d–d transitions of copper up to pH 11.7 with a maximum in ϵ at pH ~10 (Figure 8).

Around pH 4.5, λ_{max} and ϵ values are typical of a complex with two carboxylates.[11,17]



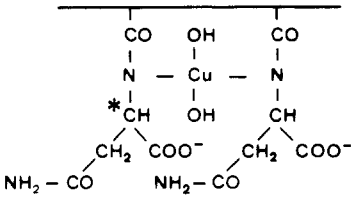
I

At pH ~7.2, the spectrum is typical of a 1:1 N:COO$^-$ complex between a carboxylate and a nitrogen of the deprotonated secondary amide group and explains the additional proton liberated at this pH (copper is able to deprotonate a secondary amide group beyond pH 5, but the deprotonation of a primary amide group is much more difficult[18]).



II

At higher pH, when a second additional proton is titrated the absortion wavelength decreases again but ϵ also decreases beyond pH ~10. This indicates that both coordination of a second nitrogen and the hydrolysis of copper–carboxylate bonds (ϵ decreases) are very likely in this pH range.



III

The differential absorption spectra in the UV range show indeed that the COO$^-$ → Cu charge-transfer transition at 260 nm is maximum at pH ~7.2 and then decreases. At the same time, the absorption increases in the range 300–350 nm, where the amide → Cu charge-transfer transitions[18–22] are located (Figure 9).

The dichroic spectra of PNMAsn:Cu in the visible range (d–d transitions) (Figure 10) show that optical activity appears near pH 5.3 when the asymmetric center becomes included in the chelate formed with two carboxylate (structure I). At higher pH, the negative band at 770 nm (B transition: $d_{xy} \rightarrow d_{x^2-y^2}$), the shoulder near 735 nm (E_b transition), and the positive band at 635 nm (E_a transition) are typical of a 1:1 N:COO$^-$ complex (structure II). Beyond

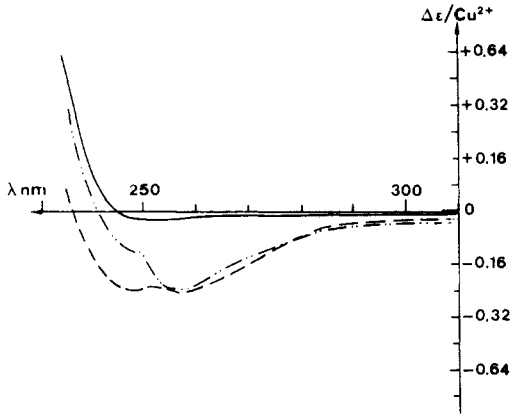


Figure 7. CD spectra of NIBAsn:Cn, R = 8, in the UV range as a function of pH: (-··) pH 4.5; (---) pH 6.03; (—) NIBAsn with no copper, pH 5.67.

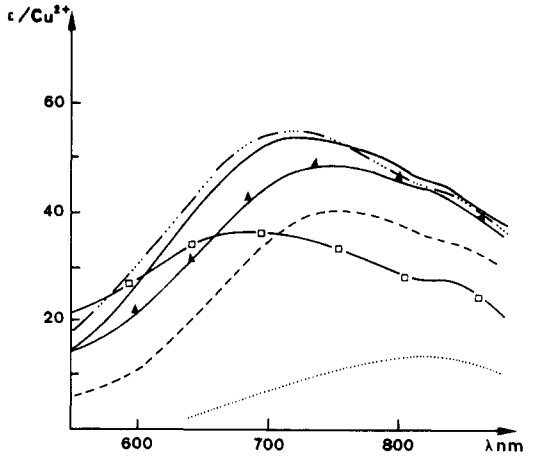


Figure 8. Visible absorption spectra of PNMAsn:Cu, R = 8, as a function of pH: (---) pH 4.5; (▲) pH 7.2; (—) pH 9.7; (-··) pH 10.2; (□) pH 11.7; (···) pure copper solution.

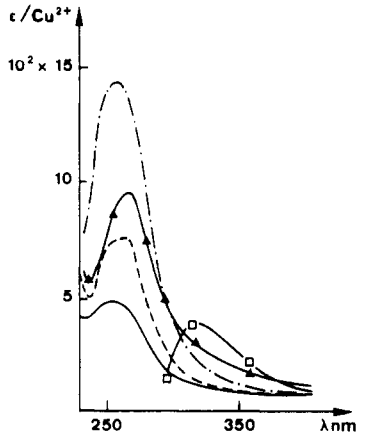


Figure 9. Differential UV absorption spectra of PNMAsn:Cu, R = 8, as a function of pH (some mixture pH 3 in the reference cell): (—) pH 6.5; (-·-) pH 7.2; (▲) pH 8–9; (---) pH 10; (□) pH 12.

pH 7, the optical activity decreases and has completely disappeared at high pH. This is in agreement with structure III, which shows that the asymmetric center is no longer included in the chelate.

The CD spectrum in the UV range at pH 3 shows only a negative band below 250 nm, corresponding to the n → π^* transition of the amide group[16] (Figure 11). At pH 5.3, a large negative band is observed between 270 and 320 nm including at least the contributions of the COO$^-$ → Cu and N amide → Cu charge-transfer transitions.[12–14,18–22] The

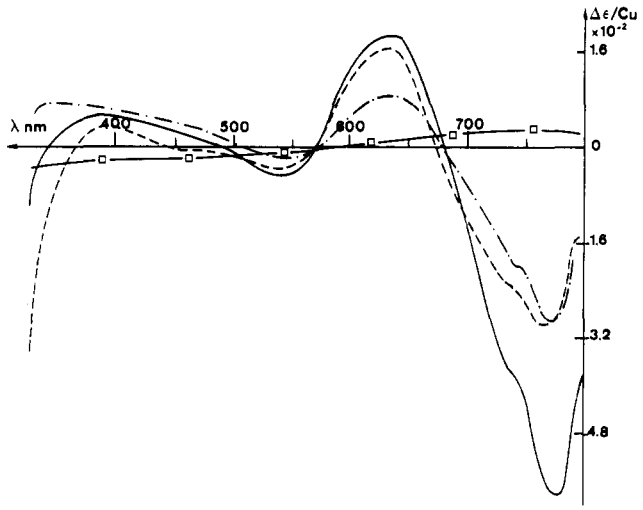


Figure 10. CD spectra of PNAMs:Cu, $R = 8$, in the visible range as a function of pH: (- - -) pH 5.3.; (—) pH 6.86; (- · -) pH 8.5; (□) pH 11.5.

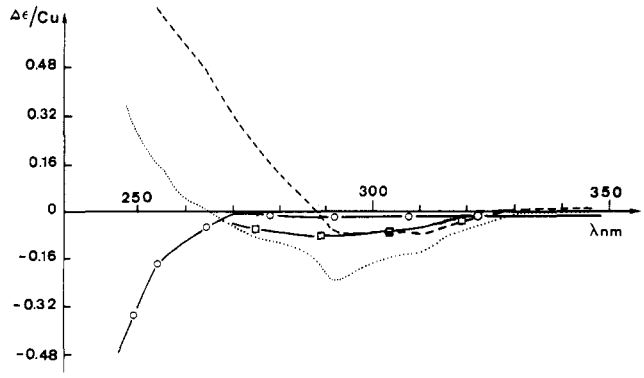


Figure 11. CD spectra of PNMAsn:Cu, $R = 8$, in the UV range as a function of pH: (○) pH 3; (···) pH 5.3; (- - -) pH 6.8; (□) pH 11.5.

sign of the n → π* transition is reversed, indicating a strong perturbation of the amide group. At higher pH the negative band decreases, because of the breaking of the COO^-–Cu bonds, but amide → Cu transitions are still present.

The results obtained with NIBAsn and PNMAsn are close to those reported for the ligands derived from alanine.[1,2] The primary amide group does not participate in complex formation. With NIBAsn, only a simple COO^-:Cu complex is formed with a low stability constant. When pH is increased, the uncomplexed copper precipitates as copper hydroxide. With PNMAsn, the vicinity of many carboxylate groups allows the formation of chelates of high stability constant, and copper is quantitatively coordinated. The high electrostatic field of the polyelectrolyte makes easier the approach of copper for complex formation. It is likely that two neighboring side chains are involved in the complex because at pH ~5 the polymer chain is fully extended due to the polyelectrolyte effect. When the pH at which the deprotonation of the amide group by copper is feasible, i.e., at pH 5–6,[18] two other complexes are successively formed (structures II and III). Thus, it can be said that the chelate with two carboxylate groups serves as an anchor for the deprotonation of the amide group, a role that is played in small molecules like peptides by a more basic function such as an amino group.[18]

It must also be pointed out that the accumulation of binding sites along the polymer chain, i.e., the high local concentration, favors the formation of 2N complexes. As shown elsewhere, polymeric ligands greatly favor the formation of bis complexes.[23,24]

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Registry No. PNMAsn (II) (homopolymer), 105502-49-6; NIBAsn (IV), 105502-47-4; isobutyroyl chloride, 79-30-1; L-asparagine, 70-47-3.

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