Polymer Bulletin 12, 141–147 (1984)

Polymer Bulletin

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The Interaction of Poly(N-Methacryloyl-L-Alanine) with Copper (II)

2. Spectroscopic Study

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Summary

The poly(N-methacryloyl-L-alanine)(PNMA):Cu system was investigated by visible and U.V. absorption spectroscopy and by circular dichroism.After formation of complex I involving only carboxylate groups,at low pH,the deprotonation of one amide nitrogen at pH c.a.5 leads to the formation of an optically active chelate ring(complex II).At higher pH,an other proton is neutralized with formation of an optically inactive complex III The structure of these complexes is discussed.

Introduction

In the previous paper it was shown that the interaction between poly(N-methacryloyl-L-alanine)(PNMA) and copper occurs in three steps beginning with the formation of a complex I in which only the carboxylate groups are involved.On further increase of the pH,the deprotonation of the side-chain amide nitrogen and/or water bound to copper occurs and leads to the formation of two other complexes.The present paper gives the results of a spectroscopic study of the PNMA:Cu system,in which the visible and U.V. absorption spectra as well as the C.D. spectra are examined.

Experimental

U.V. and visible spectra were recorded on a Cary 219 spectrophotometer,using quartz cells with 1mm and 1cm path length.
C.D. spectra were obtained from a Jobin-Yvon Mark III dichrograph flushed with dry nitrogen at room temperature.Values of $\Delta\varepsilon$ refer to copper for the visible range and to PNMA in the U.V. range.For PNMA ,concentrations are expressed in terms of the repeat unit.
Solutions with molar ratios R (PNMA/Cu) ranging from 2 to 12 were investigated.As the results were essentially similar, only the data obtained with R=4 are reported thereafter.

Results and discussion

Visible and U.V. absorption spectra

As shown in Fig.1,increase of the pH results in a decrease of the wavelength of maximum absorption for the d-d transition of copper.For PNMA in the absence of added salt,a stepwise increase of the d-d band energy indicates a two steps coordina-

tion of the ligand to copper.For the first step(λ=770-750nm) (pH 4-8) the wavelength is intermediate between the values expected for a coordination to two carboxylate groups(λ=780nm) and a coordination to one carboxylate group and one amide nitrogen(λ=700nm)(1,2).As shown in the previous paper,formation of complex II occurs before complete formation of complex I (one Cu and two COO^-).Beyond pH 8,a further decrease of λmax is observed down to a value(λ=670nm) higher than that expected for a 2N complex(complex III).
The maximum values of ε are 40 $l.mole^{-1}.cm^{-1}$ at pH 7(first step) and 27 $l.mole^{-1}.cm^{-1}$ at pH 11(second step).
For the model molecule NIBA,λmax decreases smoothly up to pH 6.5(where precipitation of copper hydroxyde occurs)indicating a coordination to carboxylate groups only.
In the presence of added salt(0.1M $NaClO_4$) the coordination process of PNMA occurs in three distinct steps(Fig.1).Formation of complex I which is much more easy in the presence of salt(see the previous paper) occurs between pH 4 and 6.5(λmax=770 nm).Complex II is formed between pH 7 and 9 with λmax=720nm. This value is in agreement with the formation of a complex in which copper is coordinated to one amide nitrogen and one carboxylate group.The theoretical value for such a complex is λ= 700nm(1) but the steric hindrance around the peptide group due to the polymer chain could account for this difference(Steric hindrance generally increases the wavelength of absorption bands for such molecules(3)).
Formation of complex III occurs beyond pH 10 (λmax=685nm).

Fig.2 shows the U.V. absorption spectra for PNMA:Cu (R=4). For PNMA alone or for the PNMA:Cu system at low pH,only a very intense band appears near 200nm corresponding to the transitions of the amide group($\pi \rightarrow \pi^*$ transitions)(3).

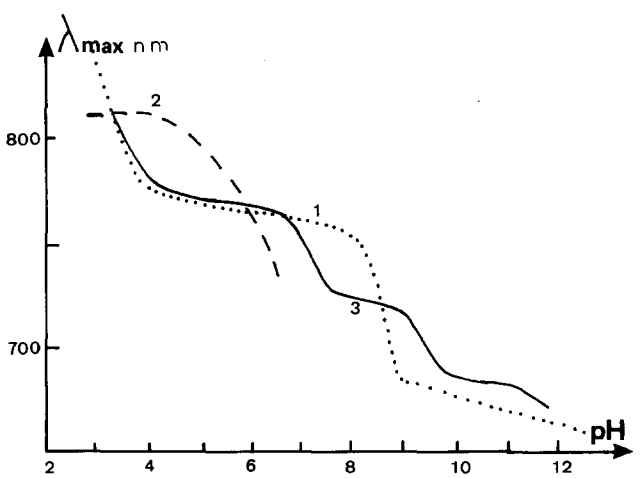


Fig.1:Variation of the maximum absorption wavelength in the visible range,as a function of pH:(1)PNMA:Cu R=4 no salt; (2)NIBA:Cu R=4 no salt;(3)PNMA:Cu R=4 in 0.1M $NaClO_4$.

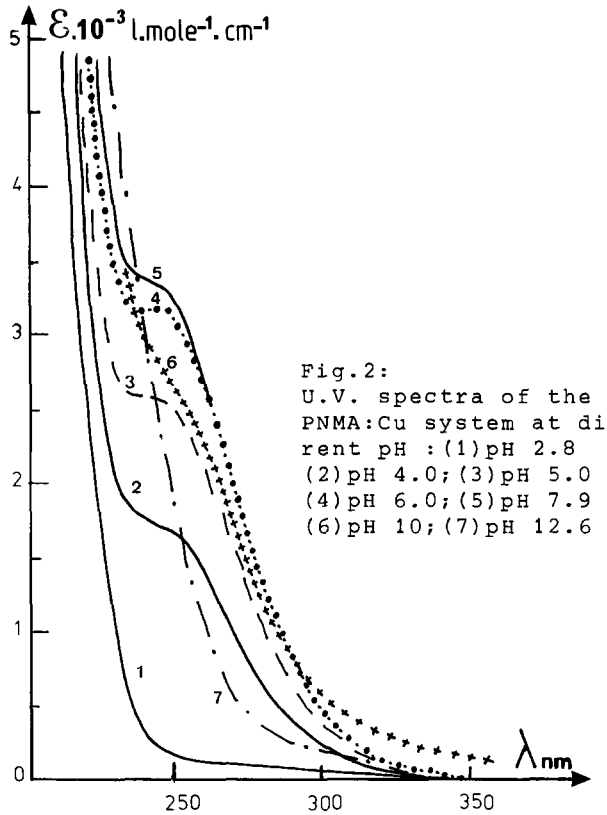


Fig.2:
U.V. spectra of the PNMA:Cu system at difrent pH :(1)pH 2.8 (2)pH 4.0;(3)pH 5.0 (4)pH 6.0;(5)pH 7.9 (6)pH 10;(7)pH 12.6

Fig.3:
Differential U.V.spectra of the PNMA:Cu system at different pH (PNMA:Cu at pH 3.8 in the reference cell):
(1)pH 7.6;(2)pH 8.6
(3)pH 11.6;(4)pH 12.0

From pH 3.5-4,an other band appears at 242nm with a maximum intensity at pH 7.9(ε/Cu=3320 l.mole^{-1}.cm^{-1}),decreasing in basic solutions.On the basis of literature data(4-9) this band may be attributed to a charge transfer transition between copper and the carboxylate group.For NIBA this band is also observed with a maximum at pH 6.5 where precipitation occurs (ε/Cu=1600 l.mole^{-1}.cm^{-1}).

Fig.3 gives the differential absorption spectra with PNMA:Cu at pH 3.9 in the reference cell and PNMA:Cu at various pH's in the sample cell.The Cu-COO CT band decreases beyond pH 7.6 and an other band appears near 305nm with an isobestic point at 292nm.The 305nm band is generally attributed to charge transfert transitions between copper and the deprotonated peptide nitrogen(10-14)

C.D.spectra

.d-d transitions:NIBA does not display any optical activity in the visible range,in the presence of copper.This is related to the fact that the only possible complex in this case is a complex involving only the COO group,excluding the existence of a chelate ring(15-17).

For PNMA,on the contrary,two C.D. bands are observed at 760 and 710nm($B_{1g}\rightarrow B_{2g}$ and $B_{1g}\rightarrow E_g$ transitions of copper respectively)(Fig.4).These bands appear at pH 4.5 and increase with a maximum at pH 7($\Delta\varepsilon$/Cu=-0.036 at 710nm and -0.030 at 760nm). No more C.D. signal is observed beyond pH 8.5.Thus only complex II is optically active.

.U.V. range:The interaction of the model molecule,NIBA, with copper leads to the perturbation of the C.D. bands located near 205nm and related with the $n\rightarrow\pi^*$ transition of the carboxyl chromophore(3)(not shown).In addition,NIBA exhibits

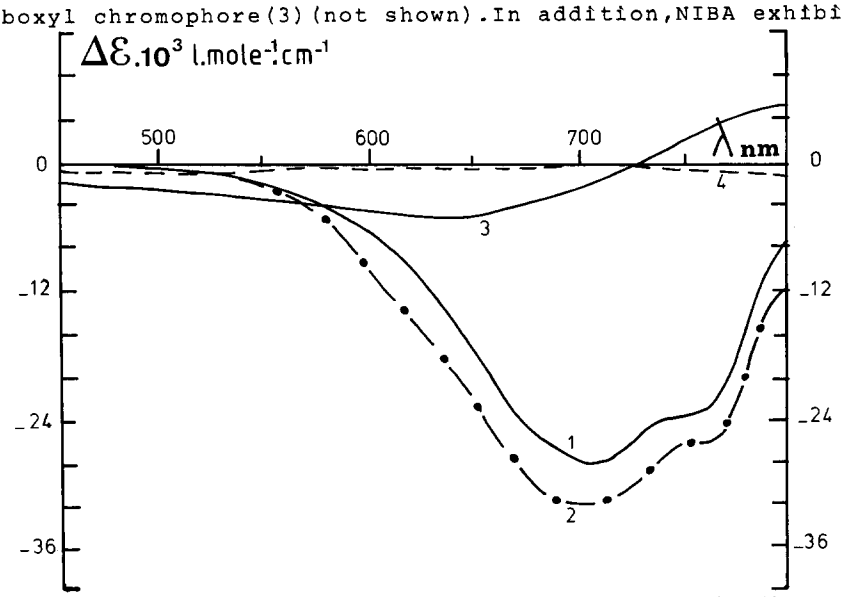


Fig.4:Visible C.D. spectra of the PNMA:Cu system (R=4) at various pH values. (1)pH 4.8; (2)pH 7; (3)pH 8; (4)pH 10 and 11.

a slightly negative band at 240nm,pH 4,in the absence of copper($n \rightarrow \pi^*$ amide(3),$\Delta\varepsilon$=-0.06).Its intensity decreases when increasing the pH(3).In the presence of copper(R=4)the same band is observed at 240nm,pH 4 with the same intensity($\Delta\varepsilon$=-0.06) but it increases up to $\Delta\varepsilon$=-0.1 when pH increases with a red shift up to 250nm.At the same time,a new band appears near 280nm related with a copper -COO interaction(7,18-20).No peptide nitrogen-Cu CT band is observed in this case.

The C.D. spectrum of copper free PNMA exhibits no band beyond 240nm(3).In the presence of copper,the range below 230 nm where are located most of the transitions of the carboxyl and amide chromophores is strongly perturbated with an inversion of the sign of the signal.Moreover a negative C.D. band appears near 250nm beyond pH 3 together with two shoulders at 280 and 315nm(Fig.5).

The origin of the 250 and 280nm bands is the same as for NIBA(CT between Cu and COO) with enhanced intensity.The 315nm band is assigned to a Cu-peptide nitrogen charge transfert(10, 14,15,20).

The nature of complexes I and II may be unambiguously deduced from the experimental results.The formation of complex I begins at low pH and involves the coordination of copper tc two(or more)carboxylate groups with the appearance of Cu-COO CT bands up to high pH values.As shown in the previous paper, this complex is very stable and exists up to high pH values. Thus,only a relatively small fraction of the ligand leads to the formation of complex II.All experimental results indicate that this is a 1N complex with a five membered chelate ring(Cu-COO and Cu-N CT bands in the absorption spectra and in the C.D. spectra).

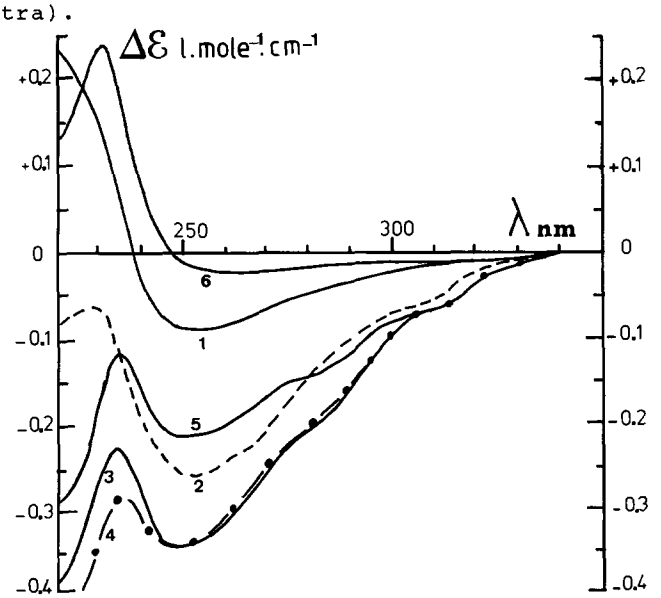
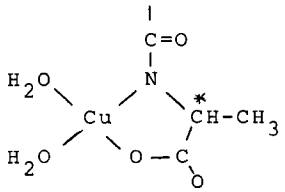


Fig.5:Ultra violet C.D. spectra of the PNMA:Cu system (R=4) at various pH values. (1)pH 3.3;(2)pH 4.1;(3)pH 5.4;(4)pH 7 (5)pH 8;(6)pH 9.



complex II

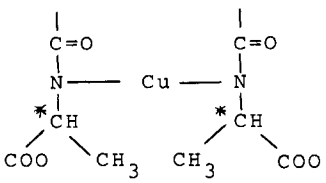
As mentionned above,the absorption wavelength of this complex is higher than that predicted by the equation of Billo(1).It may be due to the effect of the polymeric chain but in addition,this wavelength is in fact an average of the absorption wavelengthes of complexes I and II which are both present in solution between pH 4 and 9.

Concerning complex III,which forms beyond pH 9,the experimental results are more ambiguous:a second proton is neutralized for each copper ion,the Cu-COO CT bands decrease whereas the Cu-N CT band increases(Figs.2,3 and 5) and the optical activity disappears.Complex III could result from:

i)the deprotonation of a water molecule bound to copper in complex II.According to the literature,this cannot lead to a decrease of the absorption wavelength since a water molecule and a hydroxyle ion have the same contribution(1).In addition, this cannot explain the disappearance of the optical activity at high pH values.

ii)the hydrolysis of the Cu-N bond in complex II with a simultaneous deprotonation of a water molecule bound to copper in complex II(or I).This could explain the decrease of the optical activity(disappearance of the chelate)but fails to account for the increase of the Cu-N CT band.

iii)the deprotonation of an amide nitrogen in an other side-chain with breaking of the Cu-COO bonds could explain most of the results:



complex III

This is in agreement with the strong tendancy of polyelectrolytes to form the minimum number of complexes with the maximum coordination number(21).

Though the third case is the most probable,the second one cannot be excluded.It is recalled that because of the high stability of complex I,complexes II and III are only minor species and complex III derives from complex II only.

Aknowledgments

Thanks are due to Mrs M.P.Hidebrand for recording the C.D.spectra and to Dr M.H.Loucheux-Lefebvre and J.P.Aubert(Institut de Recherches sur le Cancer de Lille)for placing the dichrograph at their disposal.The authors are also greatly indebted to Dr H.Kozlowski for helpful discussions.

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Accepted July 3, 1984