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

1. Potentiometric Study

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Summary

The poly(N-methacryloyl-L-alanine)(PNMA):Cu system was investigated by potentiometry,conductimetry and Cu(II) specific measurements.After the formation of a 2:1 PNMA:Cu complex involving only the carboxylate groups,two other complexes are successively formed with additional neutralization of one and two protons per cupric ion respectively.

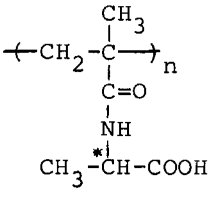
The stability constant of the first complex was determined by the Bjerrum method and was found similar to that of the poly(acrylic acid):Cu complex.

Introduction

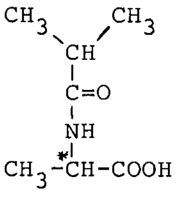
The problem of the interaction of transition metal cation with biologically active molecules is of the utmost interest (1).In recent years,many studies have been devoted to the interaction of such cations with amino-acids or peptides(2).In our laboratory we have undertaken a study of the complexation of amino-acids supported by polymeric chains(3).We present in this paper some results concerning the interaction of copper with poly(N-methacryloyl-L-alanine)(PNMA).PNMA is a water soluble polyacid and is thus expected to interact with Cu(II) in a manner similar to poly(acrylic acid) or poly(methacrylic acid) (4,5).In addition,the presence of an amide group in the side chain offers a second binding site for copper.The PNMA:Cu system was studied by potentiometry,conductimetry,visible and U.V. spectroscopy and circular dichroism(CD).The model molecule, N-isobutyroyl-L-alanine(NIBA) was also studied by the same methods.

Experimental

The synthesis and characterization of PNMA and NIBA was described in a previous paper(6).



(PNMA)



(NIBA)

Copper perchlorate $Cu(ClO_4)_2,6H_2O$(Fluka) was used as the metal ion source.Copper stocksolutions were standardized by iodometric titrations.Solutions with molar ratio R (PNMA/Cu) ranging between 2 and 8 were investigated.

pHmetric titrations were carried out with a Radiometer pHM65 pHmeter equipped with a combined electrode.The concentration of free copper in solution was determined with a Tacussel Minisis 6000 pHmeter fitted with a PCu copper selective electrode.

Results and discussion

pHmetric titrations:

The titration curves of PNMA in the absence of added salt are shown in Fig.1 for different values of R.It appears that the addition of Copper strongly lowers the pH of the solution which means that the ionization of the carboxyl groups of PNMA is easier in the presence of the metal,corresponding to the following equilibria:

$$R\text{-}COOH + M^{2+} \rightleftharpoons R\text{-}COOM^{+} + H^{+} \quad (1) \text{ complex I'}$$

$$R\text{-}COOM^{+} + R\text{-}COOH \rightleftharpoons (R\text{-}COO)_2M + H^{+} \quad (2) \text{ complex I}$$

Two pH jumps appear on the titration curves,the first one between pH 6 and 8,depending on R,the second one near pH 10.5.In the latter case,the concentration of added base C_B is given by $C_B=C_L+2C_M$ where C_L and C_M are the molar concentrations of the ligand and the metal,respectively.Thus,at pH 10.5,the formation of the complex between PNMA and Cu involves the deprotonation of the carboxyl groups and of two additional protons per cupric ion(complex III).At low R values(high copper concentration) an other pH jump is observed near pH 9 corresponding to $C_B=C_L+C_M$.

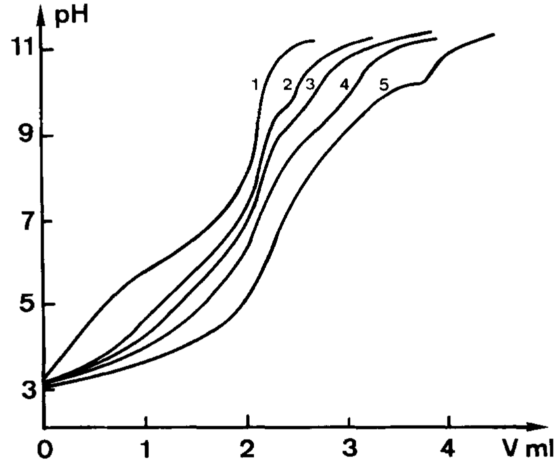


Fig.1:Titration curves of PNMA:Cu at different ratios:(1) no copper;(2):R=7.4;(3):R=5.6;(4):R=3.7;(5):R=2.2

This indicates the intermediate formation of an other complex, with the participation of the carboxyl groups and of one additional proton per cupric ion(complex II).

In the presence of added salt(0.1M KNO_3) the titration curves of the PNMA:Cu mixtures are very similar except that the depress of the pH is less important in the low pH range due to the screening effect of the salt.

The calculation of n_{H^+},the number of protons released per cupric ion,has been made at different pH as a function of R. At pH 9,n_{H^+} is equal to R+1 for R=2 and R for R=8.At pH 10.5, n_{H^+} is equal to R+2 for R=2 and R+1.7 for R=8.This assess the existence of the complexes II and III and shows that their formation is very dependent on the R value.Moreover,the variation of n_{H^+} in the low pH jump(Fig.1) suggests that the formation of complex II begins before the complete formation of complex I(Eq.(2)).For the model molecule NIBA,no effect of copper is observed on the titration curves up to pH 5.5 where the precipitation of copper hydroxyde occurs.

Measurements with a Copper selective electrode:

Fig.2 shows the variation of the fraction of free copper in the solutions as a function of pH.For the PNMA:Cu solutions the amount of free copper decreases rapidly between pH 3 and 5.5,corresponding to the formation of the $RCOOM^+$ and $(RCOO)_2M$ complexes(I' and I).At pH 7.5 no more free copper does remain in solution.Thus,the formation of complexes II and III only involves changes in the mode of binding of copper excluding the fixation of additional copper ions.For the NIBA:Cu solutions,the concentration of free copper decreases more slowly up to pH 4.5 in parallel with the formation of the $RCOOM^+$ complex(I') wich is the only one which can be obtained with NIBA in dilute solutions.Beyond pH 4.5,the concentration of free copper remains constant then decreases again at pH 6 when copper hydroxyde precipitates.

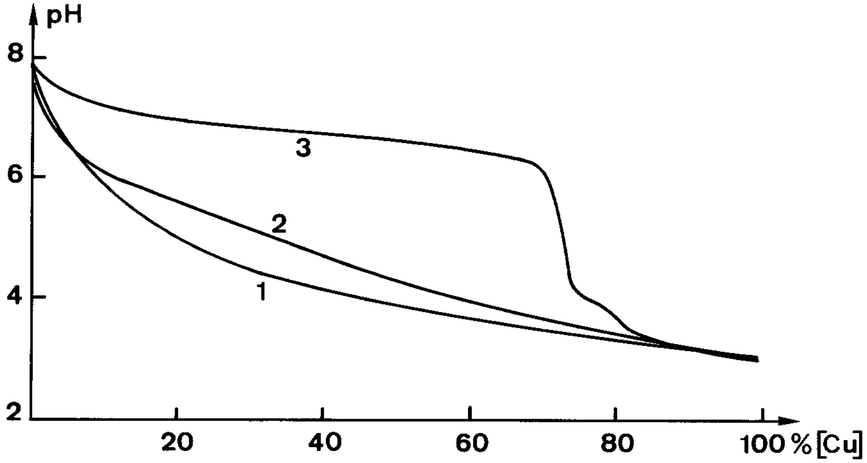
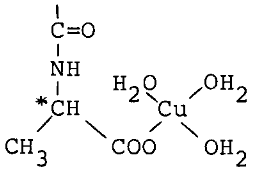


Fig.2:Variation of the concentration of free copper with pH: (1)PNMA:Cu,R=4;(2)PNMA:Cu,R=2;(3)NIBA:Cu,R=4

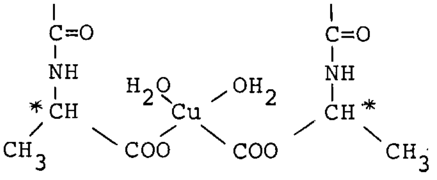
Conductimetric titrations:

The conductimetric titrations of the PNMA:Cu mixtures have been carried out at different R values. Below pH 6 the conductivity decreases then increases as expected for the titration of a weak polyacid. Between pH 7 and 9 where complex II is formed, the conductivity remains nearly constant. This is an indication that complex II is uncharged. Beyond pH 9 the conductivity increases again, suggesting that complex III is a charged one.

From the above results, it may be concluded that the interaction of PNMA with copper occurs in three steps:
First, two complexes involving only the carboxyl groups of the side chain are formed. The proposed structure for these complexes are the following:

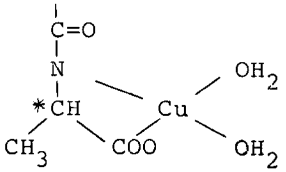


(I')



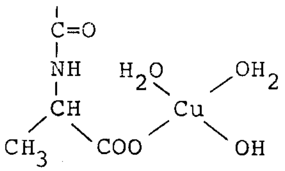
(I)

Complex I is probably stable up to high pH values, by analogy with other polycarboxylic acids. Complex I' also exists for the NIBA:Cu system.
In a second step, complex II is formed between pH 6 and 9 corresponding to $n_{H^+}=R+1$. As this complex must be uncharged, the two following structures are possible:



(II)

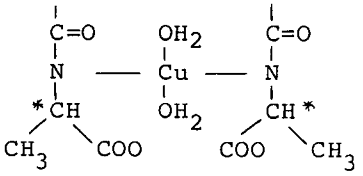
or



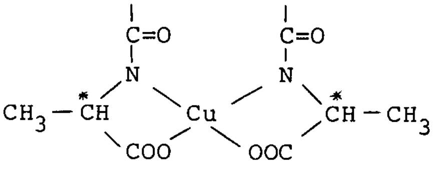
(II')

These two structures have been proposed for the copper complexes of poly(glutamic acid) (which is very similar to PNMA except that the peptide group is in the main chain) on the basis of ESR results(7). Structure II which has been demonstrated in similar systems(8) involves the formation of a very stable five membered chelate ring which follows the deprotonation of the amide nitrogen in the presence of the metal ion beyond pH 5(9).

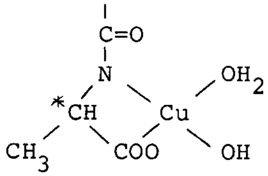
In a third step, complex III is formed near pH 10.5 ($n_{H^+}=R+2$). It could result either from the ionization of a water molecule or from the deprotonation of a second amide nitrogen in another side chain.



(III)



(III')



(III'')

The deprotonation of water molecules bound to copper in complex I is also to be considered in the high pH range. Additional data concerning the study of the d-d transitions of copper and of the charge transfer absorption bands are requested to allow a more precise attribution for the structures of these complexes. The results of such a study will be given in the following paper.

Determination of the stability constant of complex I:

The data of the potentiometric titrations curves have been treated according to the Bjerrum method(10)modified by Gregor et al.(4)for polyacids. Typical formation curves are given in Fig.3 where the average number $\bar{n}$ of ligands bound to 1 copper ion is plotted versus $p([LH]/[H^+])$. ($[LH]$ is the molar concentration of the undissociated ligand).

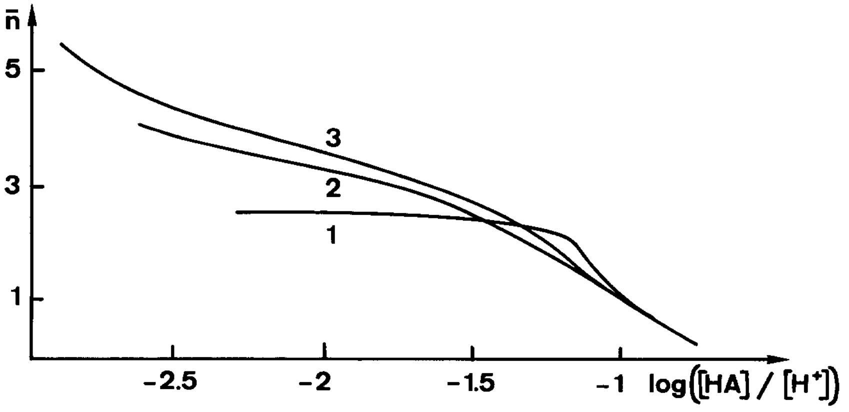


Fig.3: Formation curves for PNMA:Cu at different R values: (1) R=2; (2) R=4; (3) R=6.

When pH increases, $\bar{n}$ increases rapidly up to $\bar{n}\sim 2$ corresponding to the successive formation of complexes I' and I (equilibria (1) and (2)). Beyond this value, $\bar{n}$ increases again, more or less rapidly, depending on the value of R and indicating that the formation of complex II starts before the first one has been completed. From these curves the formation constant B_2 for the overall process:

$$2\ RCOOH + M^{2+} \rightleftharpoons (RCOO)_2M + 2H^+ \qquad (3)$$

can be determined in the usual manner (4,11) from the value of $p([LH]/[H^+])$ at $\bar{n}=1$. The constant K_2 corresponding to the following reaction:

$$2\ RCOO^- + M^{2+} \rightleftharpoons (RCOO)_2M \qquad (4)$$

may then be calculated provided that the value of the apparent ionization constant k_a of the polyacid has been determined from a Henderson-Hasselbach plot (12). Table 1 gives the values of B_2 and K_2 calculated in this way for different R values in the presence or absence of added salt.

Table 1 B_2, K_2 and k_a values for the formation of complex I in water at 25°C

	R=2 no salt	R=3 no salt	R=4 no salt	R=4 0.4M KCl	R=6 no salt
B_2	$9.5\ 10^{-3}$	$9.1\ 10^{-3}$	$7.2\ 10^{-3}$	$2.1\ 10^{-2}$	$9.1\ 10^{-3}$
k_a	$2\ 10^{-6}$	$2\ 10^{-6}$	$2\ 10^{-6}$	$5\ 10^{-5}$	$2\ 10^{-6}$
K_2	$2.4\ 10^{9}$	$2.3\ 10^{9}$	$1.8\ 10^{9}$	$8.3\ 10^{6}$	$2.3\ 10^{9}$

The value of B_2 is practically independent on R in salt-free solutions and is close to values found for other polyacids such as poly(acrylic acid) and poly(methacrylic acid) ($B_2=4.2\ 10^{-3}$ to $2.8\ 10^{-4}$; $B_2=2.5\ 10^{-4}$ respectively) (5,13).
The addition of salt increases the B_2 value which was unexpected since reaction (3) does not involve any net charge. Nevertheless it means that the formation of complex I is easier in salt containing solutions, probably through the reduction of the electrostatic repulsions between the side chains of PNMA. Upon addition of salt, K_2 decreases, on the contrary, which results from the increase of the apparent acidity constant k_a. For poly(N-methacryloyl-L-aspartic acid) (PNMAS) and poly(N-methacryloyl-L-glutamic acid) (PNMG) which are analogs of PNMA with two carboxyl groups per each residue, the value of K_2 has been evaluated to 3-5 10^{11} (14). Thus the difference in the free enthalpy ΔG for reaction (4) between PNMA and PNMAS or PNMG may be estimated to about 3 kcal/mole. This value is very clo-

se to the free enthalpy of entropic origin($\Delta G=-T\Delta S=-2.5$ kcal/mole)arising from the difference between a monomolecular and a bimolecular reaction and is explained by the fact that the formation of complex I from complex I'(reaction (2)) may be considered as a bimolecular for PNMA(i.e.involving two different side chains of the polymer) whereas it is a monomolecular one for PNMAS and PNMG(the two carboxyl groups are located on the same side chain).The same difference is found when the complexation of acetic acid and succinic acid with copper is considered(Ref.4 and references cited therein).

Conclusion

The interaction of PNMA with copper proceeds in three steps when increasing the pH.One or two carboxyl groups participate to the formation of complexes I' and I whereas complexes II and III probably involve the deprotonation of one and two amide nitrogens or water molecules.For NIBA only a type I' weak complex is formed.This is in agreement with the low stability constant of the Cu complex of N-acetylglycinate for which precipitation of the metal hydroxyde occurs before the deprotonation of the amide nitrogen is observed(15,16).
In the case of the polymer on the contrary the complexation of copper may proceed further because the carboxylate groups have a more basic character than in NIBA and thus provide a more efficient anchor for the metal ion(17).In addition, the formation of complex I prevents copper precipitation and allow the formation of further complexes.

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