

Complexation of amphoteric copolymer of 2-methyl-5-vinylpyridine–acrylic acid with copper(II) ions and catalase-like activity of polyampholyte–metal complexes

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The interaction of polyampholyte based on 2-methyl-5-vinylpyridine and acrylic acid with copper(II) ions in aqueous solution has been investigated. Several types of complexes were formed, dependent on pH. The catalytic action of polyampholyte–copper(II) complex in hydrogen peroxide decomposition has been studied. The kinetic parameters and activation energy of this process were determined. The relation between the structure of complexes and their catalytic activity is also discussed.

(Keywords: amphoteric copolymer of 2-methyl-5-vinylpyridine–acrylic acid; copper(II) complexes; catalase-like activity)

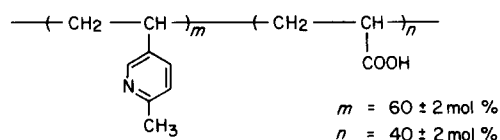
INTRODUCTION

The complexes of synthetic macromolecular ligands with transition metal ions have been widely investigated^{1–4}. In some recent publications^{5,6} the possibility is noted of modelling the complexation of metal ions with biological ligands through synthetic macromolecular systems. Among the synthetic polyelectrolytes, amphoteric copolymers most closely resemble structure and behaviour of biopolymers⁷. However, the complexation reaction between polyampholytes and metal ions has been little studied^{8–11}.

In the present paper the complexation of the 2-methyl-5-vinylpyridine–acrylic acid (2M5VPy–AA) polyampholyte with Cu^{2+} has been investigated by potentiometric and conductometric titration, u.v., e.s.r. and visible spectroscopy, and viscometry in aqueous solution. Also, the catalytic properties of polyampholyte–copper(II) complex have been studied in hydrogen peroxide decomposition.

EXPERIMENTAL

2M5VPy–AA copolymer was synthesized as in ref. 12.



It was purified by two-fold acetone precipitation from methanol solution. Copolymer composition was determined by elemental analysis and potentiometric and conductimetric titrations. The hydrodynamic-average molecular weight M_{sp} of copolymer was 2×10^5 . Potentiometric titration of 2M5VPy–AA was carried out on a OP-211/1 digital pH-meter and electroconductivity was measured on a OP-102/1 conductometer

(Radelkis, Hungary). All experiments were conducted in a thermostatically controlled cell at $298 \pm 0.1 \text{ K}$. Visible and u.v. spectra of complexes were measured on a DU-8B spectrophotometer (Beckman, USA) at room temperature. E.s.r. spectra of samples were determined on a 'Jeol ME 3X ESR' spectrometer (Japan) at 77 K . Solution viscosity was measured on a Ubbelohde viscometer at $298 \pm 0.05 \text{ K}$. Ionic strength was adjusted to the indicated value by addition of KNO_3 . Reagent-grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was used. Hydrogen peroxide decomposition was carried out in a thermostatically controlled cell at $298 \pm 0.1 \text{ K}$. pH of the solution was maintained by borate buffer ($\mu = 0.5$). The residual concentration of H_2O_2 was determined by titration of samples drawn from the reaction vessel.

RESULTS AND DISCUSSION

Structure of complexes

Potentiometric titration curves of 2M5VPy–AA at various ionic strengths μ are shown in *Figure 1*. In the presence of KNO_3 the curves are marked and have two distinct inflections. They arise from the step-by-step titration of carboxylic and pyridinium groups. The values of apparent dissociation constants pK_a determined at $\mu = 0.10$ from the dependence of $\text{pH} - \log[\alpha/(1 - \alpha)]$ on α as $\alpha \rightarrow 0$ are 3.5 and 8.0 respectively. The strengthening of acidic and basic properties of polyampholyte can be explained by inductive influence of neighbouring groups¹³.

The addition of Cu^{2+} to polyampholyte solution leads to a shift in the potentiometric titration curves, which involves formation of polyampholyte–copper(II) complex. Conductimetric and spectrophotometric data obtained in the pH interval 6.0–8.0 show that one copper(II) ion is able to bind four groups in the polyampholyte. The complex has absorption bands in the visible ($\lambda = 730 \text{ nm}$) and u.v. ($\lambda = 253 \text{ nm}$) regions (*Figure 2*). As seen from *Figure 2* the absorption maximum of

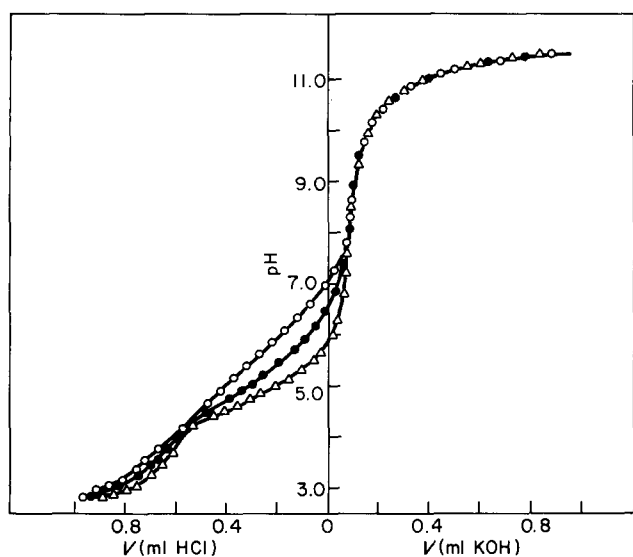


Figure 1 Potentiometric titration curves of 2M5VPy-AA in water (○) and at $\mu = 0.1$ (●) and 1.0 (△)

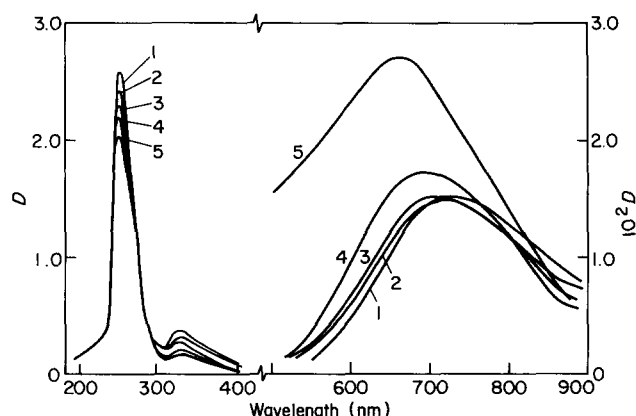


Figure 2 Visible (right) and u.v. (left) spectra of complex $[2M5VPy-AA]/[Cu^{2+}] = 4:1$ in water at $\mu = 0.1$ and pH = 7.0 (1), 8.0 (2), 9.0 (3), 10.1 (4), 11.5 (5)

2M5VPy-AA in the visible region shifts to the shorter wavelengths with increase of pH, which indicates a change in the environment of the metal ions. A similar pH-dependent visible shift for the poly(4-vinylpyridine)- Cu^{2+} system has been explained by formation of different types of complex¹⁴.

The degree of simultaneous participation of $-COOH$ and $-N=$ groups of 2M5VPy-AA in complexation was elucidated from potentiometric titration and e.s.r. data. Treatment of potentiometric titration curves by the Gregor method¹⁵ revealed the existence of two types of complex (Figure 3). One, with average coordination number $\bar{n} = 2$ and stability constant $\log \beta = 7.1$, is stable in the acidic region; in fact, the formation of this type of complex is complete at $pH \leq 6.0$. The other, with $\bar{n} = 4$ and $\log \beta = 12.0$, begins to form at $pH > 6.0$. As the major proportion of pyridine groups is ionized in the acidic region the coordination sphere of the former complex will consist of only two copolymer carboxylate groups and the complex will have the composition $Cu(COO)_2$. The deprotonization of $-NH=$ takes place at high pH and, consequently, the probability of entry of pyridine groups into the coordination sphere of copper(II) is increased. Taking into account that $\bar{n} = 4$ completion of the coordination sphere of Cu^{2+} by pyridine groups and the

formation of the complex $Cu(COO)_2(Py)_2$ can be assumed. To confirm this the e.s.r. spectrum of polyampholyte-copper(II) complex was recorded in the pH interval 4.3–11.0 (Figure 4). Analysis of the spectra indicates the existence of at least two types of complex that are distinguished one from another by parameters g_{\perp} , g_{\parallel} and A_{\parallel} . One, with $g_{\perp} = 2.058$, $g_{\parallel} = 2.334$ and $A_{\parallel} = 136 \times 10^{-4} \text{ cm}^{-1}$, corresponds to an a-type complex, which is stable in the acidic region but the line intensity decreases when the pH of the solution is increased. On the other hand the intensity of the signal of b-type complex, with parameters $g_{\perp} = 2.035$, $g_{\parallel} = 2.298$ and $A_{\parallel} = 157 \times 10^{-4} \text{ cm}^{-1}$, begins to increase in the interval $pH = 6.0\text{--}7.5$. The parameters of the a-type spectrum are close to those of poly(acrylic acid)-copper(II) and copper(II) acetate¹⁶. Therefore in the acidic region copper(II) ions coordinate with two carboxylic groups of the polyampholyte. Analogously¹⁷ the b-type spectrum with the smaller value of g_{\parallel} and larger value of A_{\parallel} can be ascribed to copper(II) ions surrounded by two carboxylate and two pyridine groups from the polyampholyte. Also, on the assumption that the structure of the complex is $Cu(PAA)_2(P2M5VPy)_2$ the values of $A_{\parallel} = 155 \times 10^{-4} \text{ cm}^{-1}$ and $g_{\parallel} = 2.310$ calculated from the parameters of individual polymer-metal complexes $PAA-Cu^{2+}$ and $P2M5VPy-Cu^{2+}$ are in good agreement with characteristics of the b-type spectrum. These results show that at $pH < 6.0$ the coordination sphere of copper(II) consist of two carboxylate groups of polyampholyte and at $pH > 6.0$ the coordination sphere is completed by two pyridine groups.

The attention should be paid to the disappearance of the e.s.r. lines of copper(II) at $pH \geq 11.0$. The behaviour of the system is reversible, e.g. the e.s.r. lines appear again when the pH falls. This phenomenon can be explained in two ways: either by broadening of spectral lines of copper(II) because of compaction of macromolecules¹⁸ or by the paramagnetic to diamagnetic transition of the complexes as a result of strong antiferromagnetic

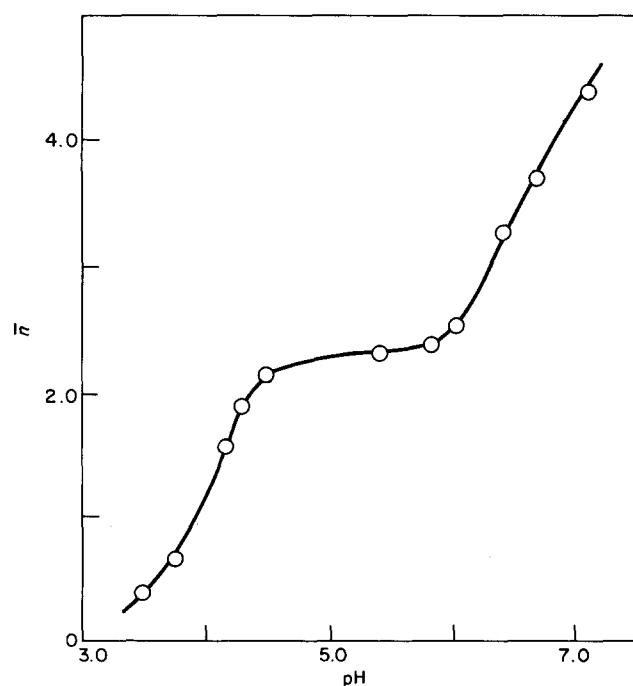


Figure 3 Dependence of the average coordination number \bar{n} on pH of the solution for polyampholyte-copper(II) complex

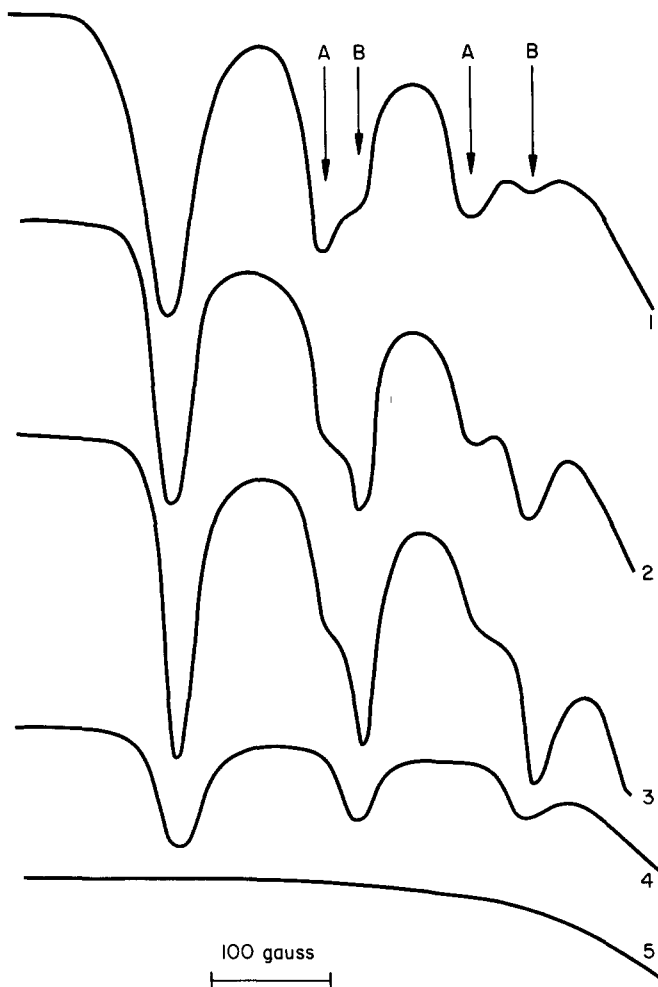
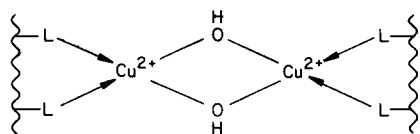


Figure 4 E.s.r. spectra of complex in water at $\mu=0.1$ and pH=4.3 (1), 5.0 (2), 6.5 (3), 8.5 (4), 11.5 (5)

coupling of the copper(II) pairs^{19,20}. As the reduced viscosity of the complex in the alkaline region is rather high the first reason can be excluded. Consequently the disappearance of an e.s.r. signal can be ascribed to the formation of binuclear complexes of the following type:

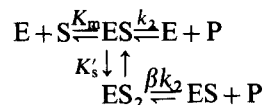


where L represents pyridine groups. It has been found²¹ that these hydroxo-bridged binuclear complexes do not exhibit an e.s.r. signal. The existence of 'e.s.r. undetectable copper' is also well known for copper-containing proteins²⁰.

Catalytic properties

The catalytic properties of the complex $[2M5VPy-AA]/[Cu^{2+}] = 4:1$ in hydrogen peroxide decomposition were studied. Decomposition curves of H_2O_2 in the presence of polyampholyte and its copper(II) complex are presented in Figure 5. Chief attention has been paid to establishing the relation between the structure and catalytic activity of the complex. As seen from Figure 5 the copolymer itself has no catalytic activity. However the rate of H_2O_2 decomposition is high in the presence of complex. The reaction is first order with respect to the substrate for $[H_2O_2]/[complex] > 20$, below this the

reaction order approximates to zero. The V_0-S_0 (where V_0 is the initial rate for H_2O_2 decomposition and S_0 is the initial concentration of H_2O_2) relation is well described by the Michaelis-Menten kinetic equation²². However increase of H_2O_2 concentration leads to inhibition of the process. Unsymmetrical character of the curve $V_0-\log[S_0]$ can be explained by additional formation of the following triple complex:



The catalytic activity of catalyst-substrate complex ES_2 is lower than that of ES ($0 < \beta < 1$). Kinetic data for the H_2O_2 decomposition reaction are presented in Table 1. Activation energy of this process calculated from the plot of $\ln K - 1/T$ is 73.2 kJ mol^{-1} . It is interesting to ascertain the influence of pH on the reaction rate of hydrogen peroxide decomposition (Figure 6). Unfortunately precipitation of the complex particles in the acidic region does not allow a complete profile of V_0-pH to be determined accurately. As follows from the e.s.r. data in the alkaline region the structure of polyampholyte-copper(II) complex is binuclear. Hence as the catalytic activity of the complex gradually increases with pH the binuclear complex is presumably responsible for the catalase-like action of the complex.

Thus the kinetic features of hydrogen peroxide

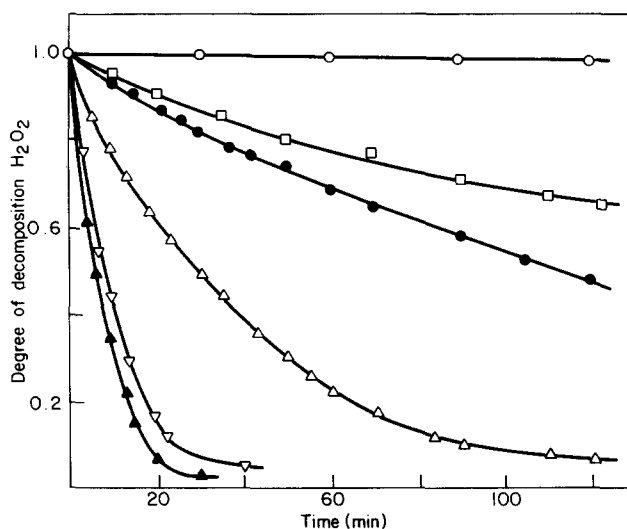


Figure 5 Decomposition reaction of hydrogen peroxide in the presence of 2M5V Py-AA (○) and complex at pH=7.0 (□), 8.0 (●), 9.0 (△), 10.0 (▽), 11.0 (▲). $[2M5VPy-AA] = 2 \times 10^{-3} \text{ mol l}^{-1}$; $[Cu^{2+}] = 5 \times 10^{-4} \text{ mol l}^{-1}$, $\mu=0.1$; $T=298 \text{ K}$

Table 1 Kinetic parameters of hydrogen peroxide decomposition in the presence of complex $[2M5VPy]/[Cu^{2+}] = 4:1$ at pH=8.50 and $\mu=0.50$

T (K)	$K_m \cdot 10^{-3}$ (mol)	$k_2 \cdot 10^{-2}$ (s^{-1})	$K'_s \cdot 10^{-2}$ (mol)	β
293	2.67	3.40	2.01	0.37
298	3.51	7.60	1.76	0.33
303	4.55	8.50	1.14	0.33
308	5.71	15.20	0.52	0.33

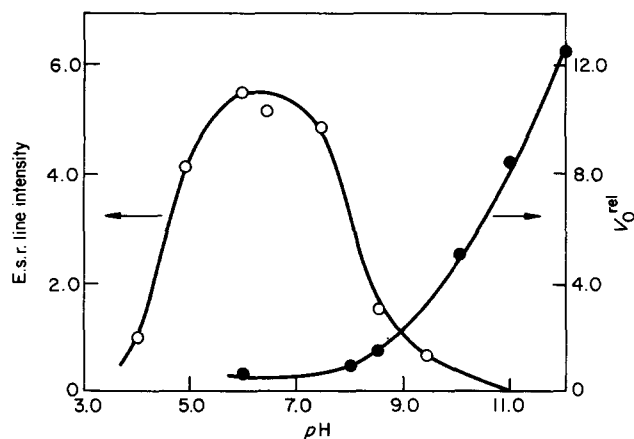


Figure 6 Dependence of initial decomposition rate of H₂O₂ (●) and change of the relative intensity of e.s.r. lines (○) upon pH

decomposition in the presence of polyampholyte-metal complex are very similar to an enzymic reaction.

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