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Research Papers

Complexation of copper(II) ions with ampicillin
I: Spectroscopic and electrochemical investigation of interactions under equilibrium conditions

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Summary

The interactions in the copper(II) ion-ampicillin system were studied by the use of electrochemical and spectroscopic methods under equilibrium conditions. In the system, two aqueous soluble coordination compounds with molar compositions of 1 : 1 and 1 : 3 and a precipitate with molar ratio 1 : 2 were formed. Copper(II) ions promoted the hydrolysis of the β-lactam group of ampicillin, therefore the ampicillin ligand in the compounds was transformed into ampicilloic acid as a result of hydrolysis. The soluble compounds are relatively stable, log K_{st} being 5.0 and 14.5, respectively. The solubility of the precipitate was constant over a broad pH range (4–10) and the solubility product constant K_{sp} of the compound was evaluated as 2.7×10^{-11} under equilibrium conditions.

Introduction

Transition metals are known to play an important role in the life of human beings. At the same time, the antibiotics which are widely used in medicine as antibacterial agents can form coordinative compounds with metal ions. Therefore, it is important to study the systems containing metal ions and antibiotics. Firstly, the formation of these coordinative compounds can have an influence on the antibacterial activity of the chemoterapeutic and can contribute to some of the side effects of antibiotic therapy. Additionally, because antibiotics are usually synthesized in metal ion-containing microbiological systems, the uncontrolled presence of metal ions can exert not only an inhibitory but also a beneficial effect on the biosynthesis (Richmond, 1981; Reiner, 1982; Helmut, 1985; Kucers and McBennet, 1988). In accordance with the above-mentioned facts, the study particular of copper(II) ion-ampicillin systems is of importance, since copper is one of the trace elements essential in nutrition and ampicillin, a β-lactam antibiotic, is widely used in medical practice. Data are available concerning the interactions between copper(II) ions and ampicillin. Schmith et al. (1967) reported a spec-

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trophotometric approach to the determination of ampicillin on the basis of its reaction with copper(II) ions. Doadrio and Garcia-Miraserra Gomez (1969) determined ampicillin spectrophotometrically by measuring the absorption of the coordination compound between copper(II) ions and ampicillin at 313–315 and 323–325 nm at pH 6.5. Veselinović and Kapetanović (1985) described a spectrophotometric study of complex formation between copper(II) ions and ampicillin. According to their investigation, ampicillin forms a coordination compound with copper(II) ions in the ratio 1 : 1 which has an absorption maximum at 316 nm.

The purpose of the present work was to study the interactions between metal ions and β-lactam antibiotics. A system containing copper(II) ions and ampicillin was chosen for this investigation and contemporary spectroscopic and electrochemical methods were applied in order to obtain reliable information.

Experimental

Materials and Methods

Ampicillin preparations in sodium salt and acidic forms were used in the investigation. Ampicillin solutions were prepared just before the experiments were performed. All reagents were of analytical grade purity. The solutions with metal-ligand molar ratio of 20 : 1–1 : 20 were prepared by pouring together aqueous solutions of ampicillin and $CuSO_4$.

Differential pulse polarographic measurements were carried out using a PAR TM 174 Polarographic analyser. The measuring system consisted of a platinum wire auxiliary and silver/silver chloride reference electrodes together with a dropping mercury electrode as a working electrode. For polarographic investigations 0.1 mol/l KNO_3 solution was used to maintain the ionic strength and/or Soerensen-Palitsch phosphate buffer (1/15 mol/l KH_2PO_4 and 1/15 mol/l $Na_2HPO_4 \cdot H_2O$) was used for all measurements, where a definite pH value was demanded. The solution in the polarographic cell was deoxygenated by bubbling with oxygen-free nitrogen.

Potentiometric measurements were carried out with a copper sulfide ion selective electrode using an MA 5704 Iskra pH meter. The content of copper was determined using a 2280 Perkin Elmer AAS spectrometer, whereas UV and visible spectra were recorded on an M40 Specord, Carl Zeiss spectrophotometer. KBr pellets were prepared from solid precipitates or sample residues obtained after rapid evaporation and drying (air stream, room temperature) of sample solutions.

Results and Discussion

Polarographic behavior

Polarographic measurements were performed at constant concentration of Cu(II) ions and increasing ligand concentrations from 1×10^{-5} to 1×10^{-3} mol/l and vice versa. The polarograms were measured for KNO_3 (pH 5.5–7.4) as well as phosphate buffer solutions. The polarograms obtained for the solution containing a constant concentration of Cu(II) ions are shown in Fig. 1. The height of peak I which corresponds to the reduction of free hydrated Cu(II) ions to Cu(0) decreases with additions of increasing amounts of ampicillin. The initial presence of ligand is demonstrated in the appearance of two new peaks which can be ascribed to the one-electron reductions of Cu(II) → Cu(I) and Cu(I) → Cu(0) (peaks II and III), respectively. Increasing amounts of ligand give rise to an additional peak (IV) which probably corresponds to the reduction of the copper bonded in the coordination compound. This peak begins to decrease after the attainment of a 1 : 1 molar ratio of metal ions to ligand, and at this time a new peak (V) appears. This peak is connected with the formation of a new coordination compound of copper with a higher content of ligand. All these peaks were observed on the polarograms of the solutions, both immediately after mixing and under equilibrium conditions. These results are similar to observations reported previously for cephalosporins, where the intermediate stabilization of Cu(I) ions also took place (Ogorevc et al., 1985).

The dependence of the i_p values for the reduction of Cu(II) ions in the presence of ampi-

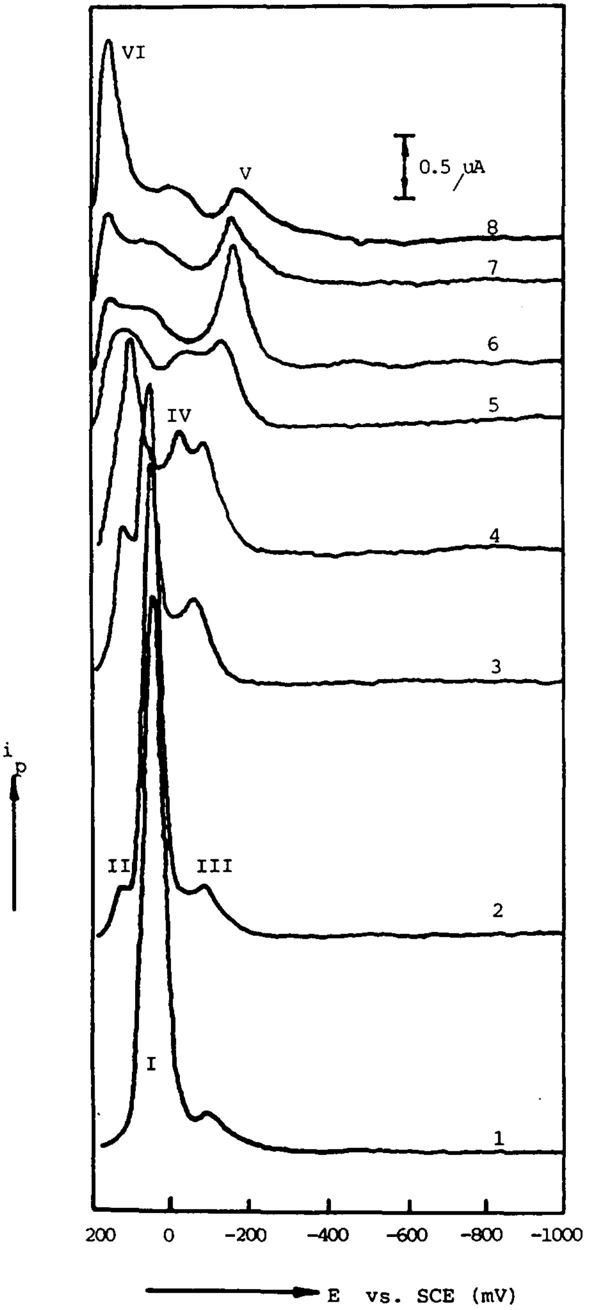


Fig. 1. DPP curves of the copper(II) ion-ampicillin system at different metal/ligand ratios: (1) 10:1, (2) 5:1, (3) 2:1, (4) 1:1, (5) 1:2, (6) 1:3, (7) 1:5, (8) 1:10. $[Cu^{2+}] = 2.5 \times 10^{-4}$ mol/l.

cillin is shown in Fig. 2. It can be seen that the i_p values corresponding to the concentration of copper ions (curve I) steadily decrease with increased ligand concentration; moreover, curves II and III indicate the stepwise reduction of copper ions.

On the other hand, the appearance of peaks IV and V (Fig. 1) can be related to the formation of the soluble coordination compounds. The maximum of curve IV suggests the molar ratio for the first coordination compound to be 1:1 and the disappearance of the corresponding peaks in the polarogram at higher concentrations of ligand must have resulted in competing reactions, e.g., in the formation of a precipitate or a new soluble coordination compound. Indeed, at a molar ratio of 1:2, the concentration of copper bonded in the coordination compound reached its minimum; afterwards, with increasing ligand concen-

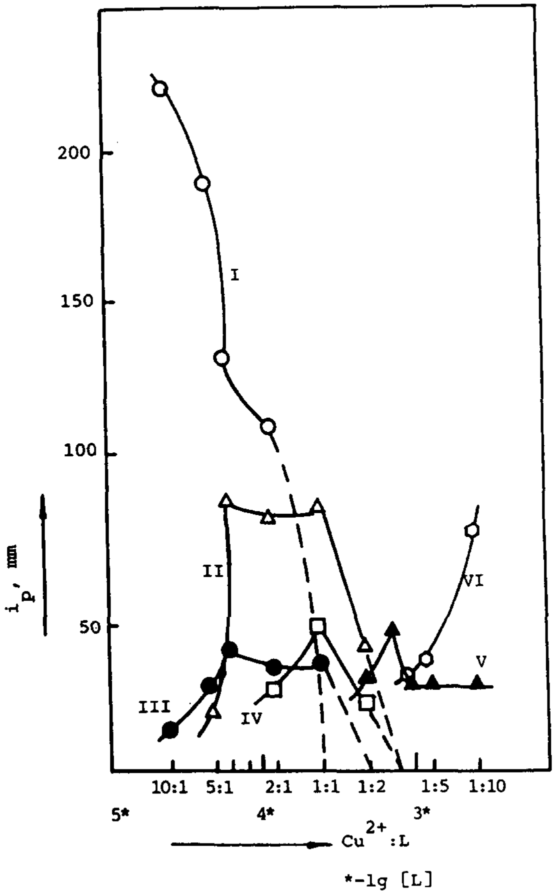


Fig. 2. Dependence of peak currents of the copper(II) ion-ampicillin system at different metal/ligand ratios. Roman numerals relate to the peaks shown in Fig. 1 (experimental parameters as in Fig. 1).

tration, the concentration of copper ions again increased, attaining its maximum at a molar ratio of 1 : 3 (curve V). On the basis of the above, one can conclude that, firstly, a precipitate of composition 1 : 2 and, subsequently, a soluble coordination compound of composition 1 : 3 are formed. The decrease in the peak with further increase in ampicillin concentration can be ascribed to the adsorption of excess ligand on the surface of the mercury drop. Moreover, it is obvious that at a ratio of Cu(II) to ligand of 1 : 2, both coordination compounds are present in solution. Consequently, at lower copper concentrations, only the initial 1 : 1 coordination compound and at higher copper concentrations, the second 1 : 3 coordination compound are present in solution. The appearance of peak VI (Fig. 1), corresponding to ampicillin at higher ligand concentrations, is in accordance with the above. From curve VI in Fig. 2, it can be seen that the peak appears at excess ligand concentration and the corresponding current increases with increasing ligand concentrations.

In the paper by Fogg and Farud (1980), devoted to the polarographic study of ampicillin, this compound was reported to be inactive in the potential range from 0.0 to −1.6 V vs SCE, whereas at the same time a peak at −0.1 V vs SCE was observed in phosphate buffer at pH 7.4. We investigated the behavior of ampicillin in the range from −0.2 to 0.2 V vs SCE and observed a peak at −0.1 V vs SCE in the same buffer solution. A more detailed study of this peak suggests that it has an anodic character and could be related to the reaction

$$n\mathrm{Hg}^0 + m\mathrm{Amp}^- \rightarrow [\mathrm{Hg}_n(\mathrm{Amp})_m]^{(2n-m)} + 2ne^-$$

Analogous behavior was also found in KNO_3 and phosphate buffer at different pH values from 6.0 to 7.4. In the case of KNO_3 the peaks were sharper and to some extent were shifted to positive potentials.

Spectroscopic investigations

The spectroscopic study of solutions containing copper(II) ions and ampicillin (sodium salt) in the visible region indicates the presence of a compound with an absorption maximum at 735 nm. The absorbance at this wavelength increases with increasing ligand concentration up to a Cu/ligand molar ratio of = 1 : 1 and subsequently falls rapidly. The reason for this is the formation of the solid compound in the system. After reaching a molar ratio of 1 : 2, the absorption peak in the ultraviolet region at 355 nm rapidly increases in intensity, concomitant with the dissolution of the precipitate. This could only result in the formation of a new soluble coordination compound. Beginning at a molar ratio of 1 : 3, the absorbance remains practically constant, with a trend toward a slight increase over the entire concentration range investigated until a molar ratio of 1 : 10. The formation of this soluble compound could be presented by the reaction

$$\mathrm{Cu(Amp)}_x + y\mathrm{Amp}^- \rightarrow [\mathrm{Cu(Amp)}_{(x+y)}]^{y-}$$

On the basis of spectroscopic and polarographic investigations, it can be concluded that in the copper(II) ion-ampicillin system, two soluble coordination compounds with molar composition at ratios of 1 : 1 and 1 : 3 are formed. Additionally, an insoluble compound of intermediate composition can be present in the system (Fig. 3). The presence of a coordination compound corresponding to the wavelength 320 nm was reported

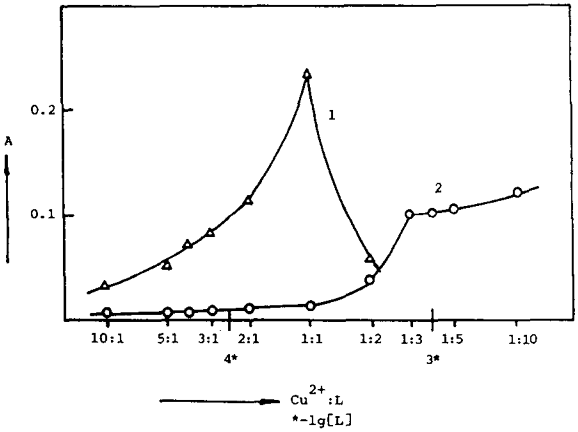


Fig. 3. Dependence of absorbance of coordination compounds in the copper(II) ion-ampicillin system; (1) at 735 nm, (2) at 320 nm.

by Veselinović and Kapetanović, (1985) and was ascribed to a compound of composition 1 : 1. The afore-mentioned authors obtained no evidence for the presence of the coordination compound having an absorption peak at 735 nm nor did they observe any indication of the precipitate. The reason for the absence of both the coordination compound and the precipitate in the study of the above workers could be that the spectrum of the solution was recorded under conditions where a considerable excess of ligand was present (1 : 5), which would yield the 1 : 3 coordination compound only and would prevent the formation of the precipitate. Furthermore, conditions such as those would be acidic and would have resulted in the anion concentrations being insufficient to allow the precipitate to form.

Ampicillin as a drug is available in acidic form or as a sodium salt. Dissolution of the latter form is accompanied by hydrolysis, which leads to the resultant solution being slightly alkaline and the antibiotic being present in anionic form.

Under these conditions, an unstable coordination compound of blue-violet color is formed owing to the bonding of donor -NH_2 groups of ampicillin with the Cu(II) acceptor orbitals. Evidence in support of this contention could be the similarity of the observed absorption spectrum with that of $Cu(NH_3)_4^{2+}$. In the case of the acidic form of ampicillin, the initial unstable blue-violet compound cannot form, since the side chain -NH_2 group is blocked by zwitterion formation (see structural formula). Additionally, ampicillin in acidic form is a relatively weak acid and hence the concentration of its anions in the solutions is relatively low. As a result, an acidic solution of ampicillin at a given concentration of copper(II) is less intensely colored than the alkaline form. Increasing pH of the acidic solution favors a shift in the chemical equilibria for complex formation as well as the precipitation of the solid compound. This can be observed on application of the drug in acidic or sodium salt form. No difference is found in the behavior of systems containing ampicillin in either the acidic form or sodium salt at pH values above 7.

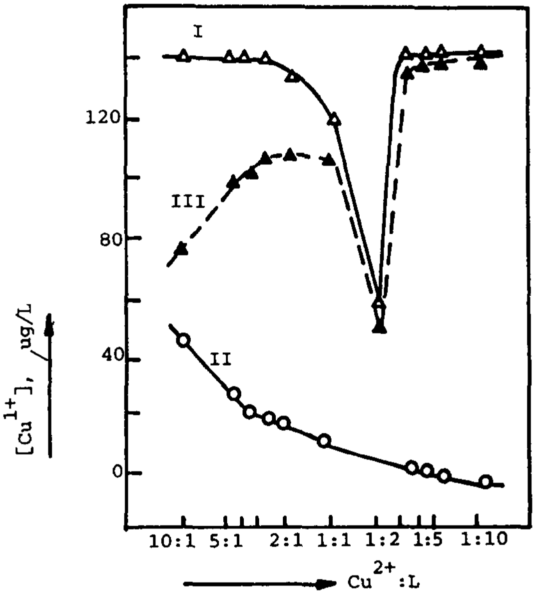


Fig. 4. Concentrations of copper in the solution of the copper(II) ion-ampicillin system under equilibrium conditions. (1) Total; (2) ionic form; (3) in coordination compounds.

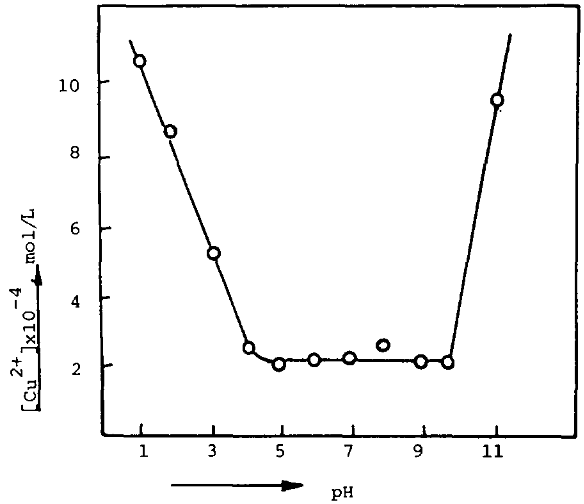
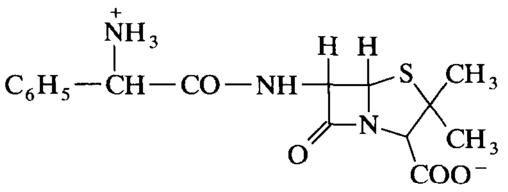


Fig. 5. Dependence of the solubility of the precipitate in the copper(II) ion-ampicillin system on pH.



Characterization of the precipitate

In order to obtain more information about formation of the precipitate in the copper(II) ion-ampicillin system, we first monitored the changes in total and copper(II) ion concentrations during the addition of an ampicillin solution to a solution containing a constant concentration of copper(II) ion. The results are presented in Fig. 4. The total concentration of metal in the solution under equilibrium conditions remains practically constant in the case of excess copper as well as excess ligand concentration. At a molar ratio of metal to ligand of 1 : 2, the total concentration of copper decreases sharply and reaches its minimum. It may therefore be concluded that the composition of the precipitate corresponds to the formula CuL_2. This conclusions was confirmed by determination of the copper concentration in the solid substance. This was found to be $8.8 \pm 0.7\%$ Cu, corresponding closely to the theoretical value of 8.4% Cu for a 1 : 2 molar ratio of Cu : L. Additionally, the difference between total copper and ionic Cu^{2+}, i.e., the concentration of copper in complex form, provides further proof for the existence of two soluble coordination compounds. Furthermore, IR spectra of ampicillin and its insoluble compound were recorded. The band at 1780 cm^{-1} corresponding to the β-lactam group (Nakamoto, 1978; Ogorevc et al., 1985) in the IR spectrum of the precipitate investigated immediately after pouring together copper(II) ions and ampicillin was observed, whilst it was absent in the spectrum of the precipitate obtained at equilibrium. Therefore, the first compound consists of the anion of the ligand ampicillin, the second being the anion of ampicilloic acid. The solubility of precipitates, isolated from the Cu(II) ion-ampicillin was studied over a broad pH region (Fig. 5). In the region pH 4–10 the solubility was minimal. The solubility product constant calculated on the basis of total copper determination at pH 7.0 was estimated to be 2.7×10^{-11} for the precipitate under equilibrium conditions and 4.9×10^{-11} for the precipitate formed immediately after precipitation (pH 7.0, 0.1 M KNO_3).

Acknowledgement

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