ELSEVIER

International Journal of Pharmaceutics 148 (1997) 191–199

international journal of pharmaceutics

Spectroscopic and polarographic investigations: copper(II)-penicillin derivatives.

A. Sher [a], M. Veber [a,*], M. Marolt-Gomiščck [b]

[a] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, P.O.B. 537, SLO-1001 Ljubljana, Aškerčeva 5, Slovenia*
[b] *Faculty of Medicine, University of Ljubljana, P.O.B. 537, SLO-1001 Ljubljana, Aškerčeva 5, Slovenia*

Received 8 August 1996; received in revised form 2 December 1996; accepted 12 December 1996

Abstract

Copper(II) derivatives of penicillins (benzypenicillin, phenoxymethylenepenicillin, ampicillin, amoxycillin and carbenicillin) have been prepared. Copper(II) ions promote hydrolysis of penicillins (L) to corresponding penicilloic acids (L*) owing to β-lactam group. Coordination compounds isolated under equilibrium conditions showed stoichiometries of the type CuL*, CuL_2^*, and CuL_3^*. Two compounds with molar metal to ligand ratio 1:1 and 1:3 are aqueous soluble and interactions at molar ratio 1:2 result in precipitate formation. The solubility of the precipitates undergoes no changes in a broad pH region. It takes place only in highly acidic or alkaline media. The infrared spectra suggest that penicillins behave as monoanionic bidentate ligands coordinating the Cu(II) ion through the carboxylic group. © 1997 Elsevier Science B.V.

Keywords: Penicillin G; Penicillin V; Ampicillin; Amoxycillin; Carbenicillin; Copper; Coordination compounds

1. Introduction

Penicillins and other β-lactam antibiotics are the most useful and least toxic of the antibiotics. In clinical praxis, benzylpenicillin, amoxicillin and phenoxymethylpenicillin are mostly applied. Recent advances have resulted in compounds with favourable antimicrobial and pharmacological properties (Bush et al., 1995). They function as a broad spectrum antibiotics due to their ability to inhibit the protein synthesis in bacteria on the ribosomes by causing misreading of the genetic code (Egorov, 1985). Copper(II) ions as biometal ions act as constituents of enzymes and other biologically active molecules. One of the roles of copper(II) as a transition metal ion is to organize structure and activate certain enzymes involved in transfer of genetic information from DNA (Sigel, 1985).

* Corresponding author. E-mail: marjan.veber@uni-lj.si

PII S0378-5173(96)04844-2

In the human body penicillins can interact with metal ions, which are present as free ions or coordinatively bounded to proteins, enzymes, amino acids, nucleic bases, nucleosides, nucleotides and other bioligands. As a result the alteration of antibacterial activity of antibiotics as well as physiological activity of copper(II) ions can take place. On the other side the knowledge of the interactions between metal ions and antibiotics is of great importance because these reactions can influence processes of the biosynthesis of antibiotics (Davies and Abraham, 1974; Page, 1984; Kucers and McBennet, 1988).

The formation of water soluble complexes with molar ratio 1:1 as a result of interactions between Cu(II) and penicillins has been reported by several authors (Cressman et al., 1966; Niebergall et al., 1966; Tomida and Schwartz, 1983; Veselinović and Kapetanović, 1985). Combined potentiometric and spectrophotometric study indicates the formation of complexes with metal to ligand molar ratios 1:1 and 1:2 (Mukherjee and Ghost, 1991).

In our previous investigations (Sher et al., 1993a,b) electrochemical and spectroscopic techniques were applied for the characterization of water soluble as well as insoluble complexes that are formed in aqueous solutions between Cu(II) ions and ampicillin.

The purpose of the present work was to expand the investigations on other antibiotics of penicillin group and to study the formation of soluble complex compounds as well as the slightly soluble compounds, which are formed in the systems containing copper(II) ions and penicillins.

2. Experimental

2.1. Materials

Benzylpenicillin—penicillin G, amoxycillin, carbenicillin (Pliva, Zagreb), ampicillin and phenoxymethylenepenicillin—penicillin V (Lek, Ljubljana) were used in the investigation. Penicilloic acids were prepared by hydrolysis of particular penicillins. All reagents used were of analytical purity.

2.2. Apparatus

Differential pulse polarographic measurements were performed using a PAR TM 174 polarographic analyzer. The measuring system consisted of platinum wire counter, Ag/AgCl reference and mercury dropping electrode as a working electrode.

A 2280 Perkin Elmer atomic absorption spectrometer was used for the determination of total metal concentration at equilibrium conditions.

UV and visible spectra were recorded on a M40 Specord Carl Zeiss spectrophotometer.

Infrared spectra were recorded with 727B Perkin Elmer IR spectrometer using KBr pellets technique. A Janetzki T32c centrifuge and an ultrasonic stirrer 2R Iskra were applied in the characterization of slightly soluble compounds.

2.3. Procedures

Polarographic and spectroscopic measurements were performed in solutions either at constant copper(II) concentrations and variable ligand concentrations in the concentration range from 1×10^{-5} to 1×10^{-3} mol/l or vice versa at constant ligand and variable metal ion concentration. The molar ratio metal to ligand was changed from 10:1 to 1:10. The polarograms were recorded in phosphate buffer at pH 7.5 as well as in 0.1 M KNO_3 at pH 6.5–7.7. The interactions were followed under the equilibrium conditions and immediately after the mixing of solutions of Cu(II) and antibiotic.

In order to study the precipitation of slightly soluble compounds aqueous solutions of copper(II) ions and antibiotics in the molar concentration ratios from 10:1 to 1:10 were poured together. The experiments were performed either at constant concentrations of copper ions (2.5×10^{-4} mol/l or 5×10^{-4} mol/l) and variable ligand concentrations or vice versa. In this case the concentration of antibiotic was kept constant at 5×10^{-4} mol/l. The pH of the solution was adjusted with KOH or HCl to 6.8.

The precipitates were prepared by mixing $CuSO_4$ solution and particular antibiotic (molar ratio metal/ligand 1:2). The precipitate was

filtered through the filter paper (Schleicher and Schüll, Nr. 598, 'blue ribbon') immediately and after different intervals after the precipitation. They were carefully washed and finally dried on air at room temperature. For the solubility determinations small quantities of precipitates obtained were transferred into test-tubes containing 0.1 M KNO_3 and the pH of the solution was adjusted with KOH or HCl solution. The system was agitated and kept for 24, 48 or 150 h. Afterwards it was centrifuged for 30 min at 4000 revs./min. The solution was decanted and copper concentration was determined by AAS.

For the characterization of insoluble compounds the precipitate was washed, dried on the air to the constant weight and after dissolving the weighted amount of the substance in HNO_3 (1:1) the determination of copper ion concentration was carried out by atomic absorption spectrometry.

3. Results and discussion

The polarographic measurements have showed, that all investigated antibiotics behave similarly in the presence of Cu(II). Several polarographic peaks (I–V) can be observed in this system at different ligand to metal ratios which can be ascribed to the reduction of copper from different copper containing species (Fig. 1). The peak I can be related to the two electronic reduction of hydrated Cu(II) ion. Its height decreases with the additions of penicillin. At the same time new peaks (II, III and IV) appear. While the first two can be related to the reduction of Cu(II) to Cu(I) and Cu(I) to Cu a well-shaped peak (IV) can be ascribed to the reduction of copper bonded in a coordinative compound. Its intensity increases up to the molar ration ligand to metal 1:1. At higher ligand concentrations this peak begins to decrease but at the same time a new peak appears (V) which is related to the formation of the second soluble complex compound with higher ligand content.

The same pattern of polarograms has been observed also for all antibiotics of penicillin group investigated. Just a slight differences in peak potentials have been observed which are evident from data presented in Table 1. From these data it can be concluded that the structural changes in different penicillins do not significantly influence the reduction of copper ions in the presence of different antibiotic ligands. Clear changes of peak potentials were typical for peak (IV), especially for carbenicillin. This substance, in opposite with penicillin G, penicillin V, amoxycillin and ampicillin with $-NH_2$, and -OH electron donor groups, contains strong electron donor -COOH group.

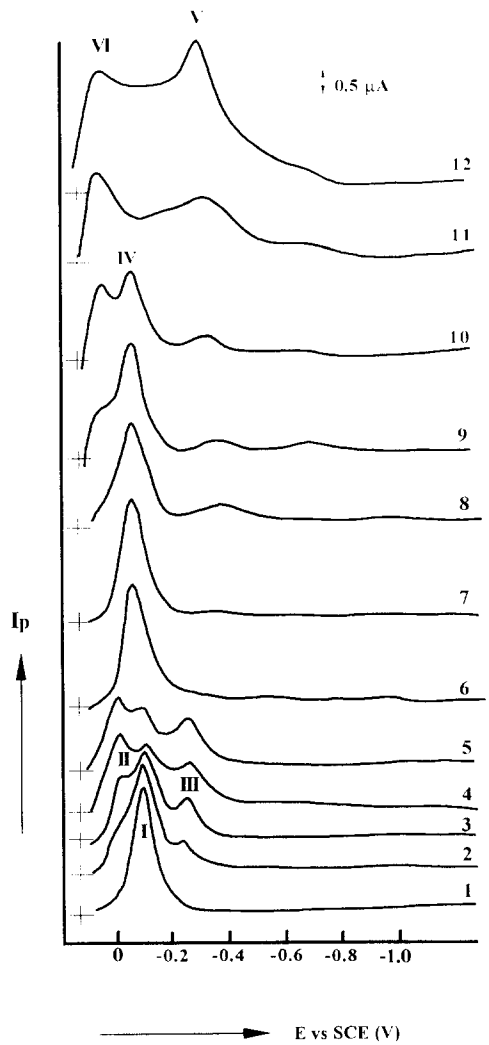


Fig. 1. DP polarograms in the system Cu^{2+}–benzylpenicillin at different metal to ligand ratio ($[Cu^{2+}] = 2.5 \times 10^{-4}$ mol/l). 1, Cu^{2+}; 2, 10:1; 3, 5:1; 4, 4:1; 5, 3:1; 6, 2:1; 7, 1:1; 8, 1:2; 9, 1:3; 10, 1:4; 11, 1:5; 12, 1:10.

Table 1
DP Polarographic reduction potentials in the system Cu(II)–penicillin

El. process	Peak potential vs. SCE (mV)						Molar ratio of coordinative compound (Cu:L)
	Peak	A	B	C	D	E	
$Cu^{2+}+2e \rightarrow Cu$	I	−100	−95	−85	−95	−90	—
$Cu^{2+}+e \rightarrow Cu^{+}$	II	−10	−50	−5	0	−10	—
$Cu^{+}+e \rightarrow Cu$	III	−275	−285	−300	−265	−305	—
$[CuL]x+2e \rightarrow Cu$	IV	−240	−175	−220	−65	−265	1:1
$[CuL]y+2e \rightarrow Cu$	V	−270	−315	−340	−300	−320	1:3

A, benzylpenicillin (PenG).
B, amoxycillin (Amox).
C, ampicillin (Amp).
D, carbenicillin (Carb).
E, phenoxymethylpenicillin (PenV).

From polarographic studies it can be concluded that at least two soluble complexes are formed in the system containing Cu(II) ions and penicillins with molar ratios 1:1 and 1:3.

Polarographic studies were completed by spectroscopic measurements. The existence of coordinative compounds can be confirmed from absorption spectra in the VIS and UV region (Figs. 2 and 3). The maxima of absorption were observed at 715–755 nm (coordination compounds with ligand to metal molar ratio 1:1) and 318–366 nm (coordinative compound with ligand to molar ratio 3:1) (Table 2). Intensity of peaks at 750 nm increases up to the ligand to metal ratio 1:1. At further addition of ligand into the solution the decreasing of absorption peak occurs which is related with the precipitate formation. The minimum of absorption at these wavelengths was observed at metal to ligand molar ratio 1:2 (Fig. 4). After further addition of ligand the absorption in UV was observed with maximum at 320 nm. This absorption is due to the formation of the second coordinative compound. Using molar ratio method the molar compositions of both coordina-

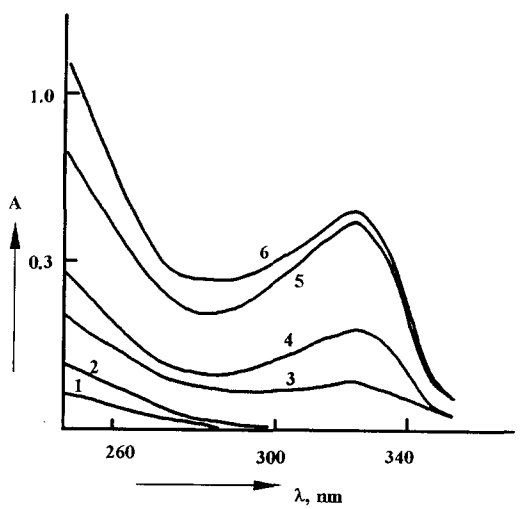


Fig. 2. UV Absorption spectra in the system Cu^{2+}–benzylpenicillin at different metal to ligand ratio (1, 1:1; 2, 1:2; 3, 1:3; 4, 1:4, 5, 1:5; 6, 1:10).

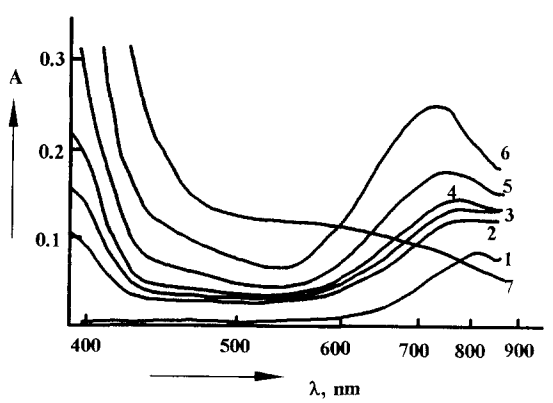


Fig. 3. Absorption spectra in the system Cu^{2+}–benzylpenicillin at different metal to ligand ratio (1, Cu^{2+}; 2, 5:1; 3, 4:1; 4, 3:1; 5, 2:1; 6, 1:1; 7, 1:2).

Table 2
Spectral data for coordinative compounds in the Cu-penicillin system

Absorption maxima (nm)					Molar ratio of coordinative compound (Cu:L)
Benzyl-penicillin	Ampicillin	Amoxycillin	Carbenicillin	Phenoxymethyl penicillin	
755	735	760	715	755	1:1
318	320	355	326	332	1:3

tive compounds were determined. They are found to be 1:1 and 1:3 respectively.

The precipitates with different colours from green to brown which are formed in the copper(II) ions–penicillin systems at some molar to ligand ratios were subject of further investigations. These studies were carried out mostly at the equilibrium conditions. To determine the solubility of these compounds the concentration of copper in the supernatant solutions was determined. It was lowest at the copper(II) to ligand molar ratio of 1:2 (Fig. 5), what indicates that the composition of the substance corresponds to this molar ratio. The chemical determination of copper content in the precipitates supported this assumption (Table 3).

IR spectra of penicillins and their compounds with copper(II) ions have also been investigated. Although a complete assignment of vibrational spectra is outside of the scope of this work, a tentative assignment of strong bands in the (C=O) region is described below. (C=O) vibrations of β-lactam, amide and carboxylic groups are expected between 1800 and 1300 cm^{-1}. The peaks at 1780 and 1680 cm^{-1} have been assigned to the C=O stretching vibration of the β-lactam and amide groups, respectively, in accordance with the

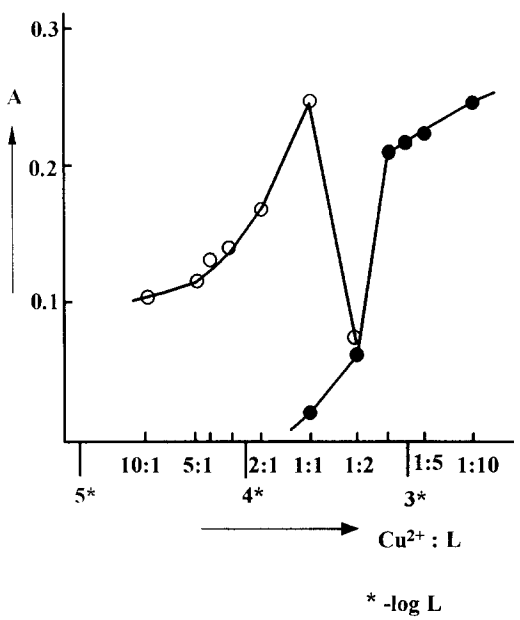


Fig. 4. The dependence of absorbance on metal to ligand ratio in the system Cu^{2+}–benzylpenicillin (○ at λ 755 nm, ● at λ 318 nm)

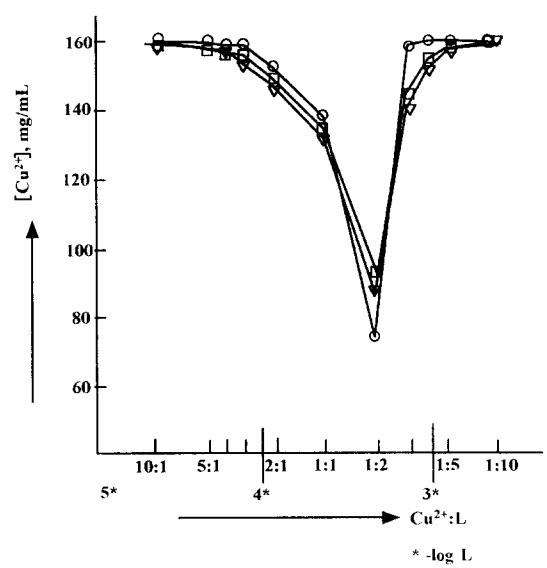


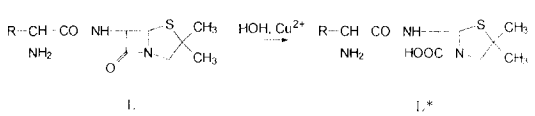
Fig. 5. The total concentration of copper(II) in the solution of Cu–penicillin systems at equilibrium conditions (▽ Cu–penicillin; ○ Cu–ampicillin; □ Cu–carbenicillin).

Table 3
The content of copper in precipitates

CuL_2	Determined (%)	Calculated (different metal to ligand ratios) (%)		
		2:1	1:1	1:2
$Cu(PenG^*)_2$	8.2	27.7	16.1	8.7
	8.3			
	6.6			
$Cu(Amp^*)_2$	7.7	26.8	15.5	8.4
	8.9			
	9.8			
$Cu(Amox^*)_2$	9.9	26.0	14.9	8.1
	7.5			
$Cu(Carb^*)_2$	8.7	25.3	14.5	7.8
	6.8			

data in the literature (Nakamoto, 1978). A band at 1520 cm^{-1} is present in the spectrum of ampicillin while it was not found in the spectrum of penicillin G. The band can be assigned to the symmetrical vibrations of NH_2 group. The IR investigation of the precipitates measured immediately after the preparation showed the presence of a band at 1780 cm^{-1} which could be ascribed to the β-lactam group in the compound. This is also true for the dry precipitate after 2–3 months storing. While keeping it in the solution at above mentioned equilibrium conditions (48 and 150 h) the peak at 1780 cm^{-1} disappears indicating the hydrolysis of the group at the β-lactam group (Rapson and Bird, 1963; Mustafi and Makinen, 1995). The rate of hydrolysis increases with increasing pH of the solution. IR spectrum of the precipitate formed by pouring together the solutions of $CuSO_4$ and a penicilloic acid is analogous with the spectrum of the hydrolysed precipitate which was obtained by mixing $CuSO_4$ and penicillin solution. Consequently, at equilibrium conditions the precipitate containing a penicilloic acid as a ligand (L*) is present.

The scheme of the hydrolysis of penicillins to penicilloic acids are shown below:



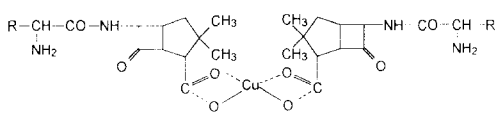
I. L*

Some IR data of penicillins and their precipitates with copper(II) ions are shown in the Table 4. One can see that the amid (1660 cm^{-1}) and the acidic (1610 and 1390 cm^{-1}) bands are shifted. The bands at 1780 cm^{-1} related to vibrations of β-lactam group are practically absent in the spectra of the copper containing penicillin compounds ($Cu(Pen^*)_2$).

Thus, the investigation of IR spectra of precipitates formed at equilibrium in the systems Cu(II)-penicillin showed that they consisted of copper ions attached to penicilloic acid (absence of the band related to β-lactam group). It is likely that Cu(II) ions promote the process of penicillin hydrolysis to penicilloic acid (Cressman et al., 1966).

The formation of the precipitates in the system of copper(II) ions with other penicillins occurs in the analogous way.

On the basis of all mentioned above it can be concluded that in the system Cu(II) and penicillin firstly the precipitate CuL_2 is formed and after that it is transformed in CuL_2^* in aqueous solutions. So it is possible to suppose that the interaction between Cu(II) ions and carboxyl group in penicillins takes place at the formation of the first precipitate, and after that the hydrolysis of the β-lactam group occurs in the ligand of the residue in the accordance with the following scheme:



It was also confirmed that the same process of interaction between Cu(II) ions and carboxylic group takes place with cephazolin (Ogorevc et al., 1985) which also belongs to β-lactam antibiotics.

The effect of pH on the solubility of copper(II) penicillin compounds in the systems Cu(II)–penicillin at equilibrium was studied. The solubility of the precipitates $Cu(Pen^*)_2$ undergoes no changes in a broad pH region (Fig. 6).

The dissolving of the precipitates takes place only in very acidic or alkaline solutions. In the case of $[Cu(Amox^*)_2]$ the formation of a blue green precipitate takes place at pH > 12.5, which can be ascribed to the alkaline salt of copper(II)

Table 4
Data of IR-spectra of penicillins and their compounds with copper(II) at equilibrium

Functional group	Wave number (cm^{-1})			
	Pen G	$Cu(PenG^*)_2$	Amp	$Cu(Amp^*)_2$
Lactamic	1780	–	1780	–
Amidic	1680	1660	1680	1660
Carboxylic	1605	1610	1605	1610
Carboxylic	1402	1395	1400	1395

or its hydroxide. The last assumption is related to the fact that the value of K_{sp} for $Cu(OH)_2$ is rather low and at high concentrations of hydroxide ions the conditions for precipitation in accordance with the solubility product constant ($K_{sp} = 1.6 \times 10^{-19}$) are fulfilled.

The same character of the formation of the precipitates as well as their similar behaviour in the wide pH range indicates an insignificant effect of the side chain (R) on the bonding of ligand with Cu(II) ions.

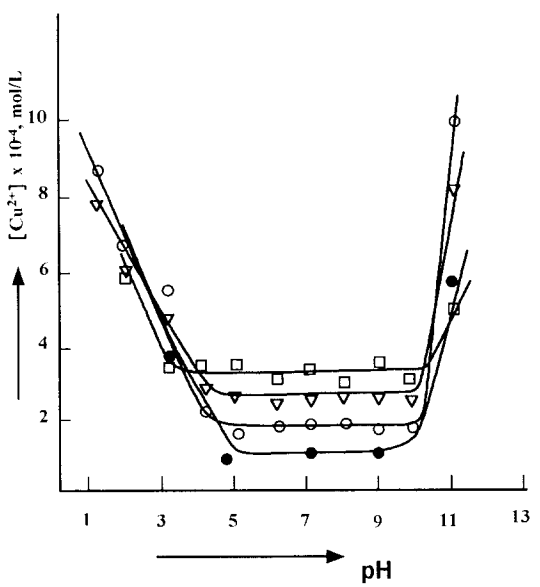


Fig. 6. The dependence of the solubility of the precipitates on pH in penicillin systems: (▽ Cu–penicillin; ○ Cu–ampicillin; □ Cu–carbenicillin: ● Cu–amoxycillin).

It is obvious from the data in the Table 5 that the K_{sp} values of precipitates differ insignificantly. This fact also indicates the similarity of the bond character in the copper containing compounds of penicillin antibiotics. At the same time the solubility of compounds increases insignificantly in the order penicillin G → ampicillin → amoxycillin. This is probably related to the strengthening of the bond as a result of the additional influence amine donor group of ampicillin and amine and hydroxyl groups of amoxycillin in comparison with penicillin G.

Some increase of the K_{sp} value for $Cu(Carb^*)_2$ can be ascertained to the presence of the additional acceptor carboxylic group of carbenicillin in comparison with penicillin G. This causes the decrease of the bond strength in comparison with the interaction between Cu(II) ions and penicillin G.

The interaction of Cu(II) ions with all antibiotics of penicillin group can be described in the same way. At the excess of metal the soluble complex with the composition 1:1 (Cu/L) appears, which is changed to CuL* owing to the hydrolysis of the ligand. The precipitate CuL_2 formed at molar ratio 1:2 is also transformed into CuL_2^* in aqueous solutions at equilibrium. In solutions containing the excess of metal the precipitate dissolved and the complex with ligand to molar ratio of 1:3 is formed. On basis of IR spectra it can be assumed that in the solid compounds the site metal ion coordination involves the carbonyl oxygen and it does not involve β-lactam carbonyl oxygen. Therefore the following processes can be proposed which can be generalised for all antibiotics of penicillin group:

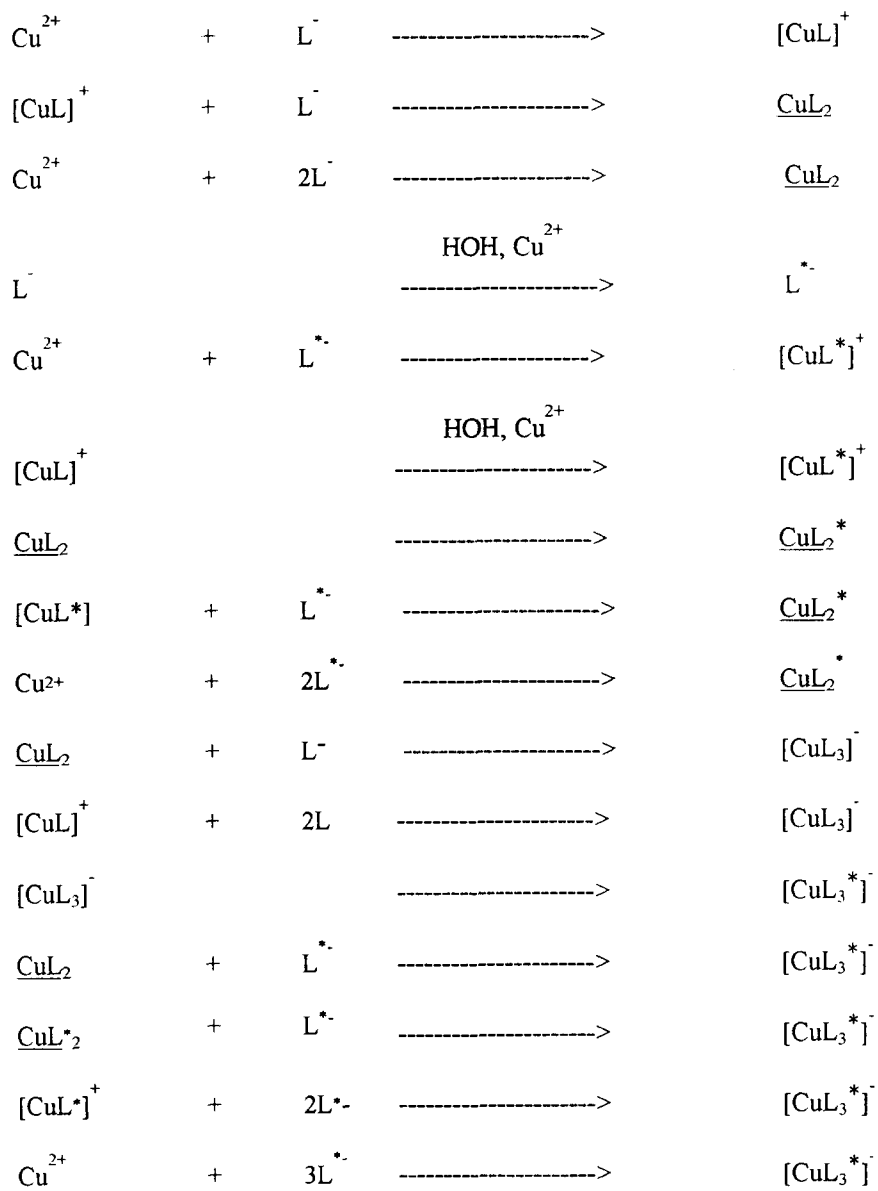


Table 5
The solubility product constants for compounds between Cu(II) and penicillins

CuL_2^*	Cu^{2+} (mol l^{-1})	Solubility (g/100 ml)	K_{sp}	$-\log K_{sp}$	Region of pH with constant solubility
$Cu(PenG^*)_2$	2.8×10^{-4}	2.1×10^{-2}	8.8×10^{-11}	10.1	4.0–10.0
$Cu(Amp^*)_2$	1.9×10^{-4}	1.5×10^{-2}	2.7×10^{-11}	10.6	4.0–10.0
$Cu(Amox^*)_2$	1.0×10^{-4}	8.3×10^{-2}	4.0×10^{-12}	11.4	4.5–9.5
$Cu(Carb^*)_2$	3.1×10^{-4}	2.6×10^{-2}	1.2×10^{-10}	9.9	3.0–10.0

where L is penicillin ligand and L* is penicilloic acid

Acknowledgements

Financial support from the Ministry for Science and Technology of Slovenia is gratefully acknowledged.

References

Bush, L.M., Calmon, J. and Johnson, C.C., Newer penicillins and beta-lactamase inhibitors. *Infect. Dis. Clin. North Am.*, 9 (1995) 653–665.

Cressman, W.A., Sugita, E.T., Doluisio, J.T. and Niebergall, P.J., Complexation of penicillins and penicilloic acids by cupric ion. *J. Pharm. Pharmacol.*, 18 (1966) 801–815.

Davies, R.B. and Abraham, E.P., Metal cofactor requirements of β-lactamase II. *Biochem. J.*, 143 (1974) 129–135.

Egorov, N.S., *Antibiotics—A Scientific Approach*, Chpt. 8. MIR Publishers, Moscow, 1985.

Kucers, A. and McBennet, N., *The Use of Antibiotics*, Medical Books, London, 1988.

Mukherjee, G.N. and Ghost, T.K., Metal ion interactions with antibiotic drugs. III. Complex formation of copper(II) with ampicillin. *Indian J. Chem.*, 30 (1991) 1033–1037.

Mustafi, D. and Makinen, M.W., Structure, conformation and probable mechanism of hydrolysis of a spin-labelled penicillin revealed by electron-nuclear double-resonance spectroscopy. *J. Am. Chem. Soc.*, 117 (1995) 6739–6746.

Nakamoto, K., *The Infrared and Raman Spectra of Inorganic and Coordination Compounds*, Wiley-Interscience, New York, 1978.

Niebergall, P.J., Hussar, D.A., Grosman, W.A., Sugita, E.T. and Doluisio, J.T., Metal binding tendencies of various antibiotics. *J. Pharm. Pharmacol.*, 18 (1966) 729–736.

Ogorevc, B., Hudnik, V., Gomišček, S., Smyth, M.R. and Vos, J.G., A spectroscopic and polarographic investigation of the complexation of cefazolin with copper(II) ions, *Inorg. Chim. Acta* 108 (1985) 13–16.

Page, M.I., The mechanism of reactions of β-lactam antibiotics. *Acc. Chem. Res.*, 17 (1984) 144–150.

Rapson, H.D.C. and Bird, A.E., Ionization constants of some penicillins and of their alkaline and penicillinase hydrolysis products. *J. Pharm. Pharmacol.*, 15 (1963) 227–231.

Sher, A., Veber, M., Marolt-Gomišček, M. and Gomišček, S., The study of complexation of copper(II) ions with ampicillin. I. Spectroscopic and electrochemical investigations of interactions at equilibrium conditions. *Int. J. Pharm.*, 90 (1993a) 181–186.

Sher, A., Veber, M., Marolt-Gomišček, M. and Gomišček, S., The study of complexation of copper(II) ions with ampicillin. II. Dynamics of reactions. *Int. J. Pharm.*, 99 (1993b) 119–123.

Sigel, H., (Ed.), *Metal Ions in Biological Systems*, Vol. 19, Marcel Dekker, New York, 1985.

Tomida, H. and Schwartz, M.A., Further studies on the catalysis of hydrolysis and aminolysis of benzylpenicillin by metal ions. *J. Pharm. Sci.*, 72 (1983) 331–339.

Veselinović, D.S. and Kapetanović, V.P., Spectrophotometric investigation of Cu(II) ion complex with ampicilllin. J. Serb. Chem. Soc., 50 (1985) 401–405.