

A Spectroscopic and Polarographic Investigation of the Complexation of Cefazolin with Copper(II) Ions

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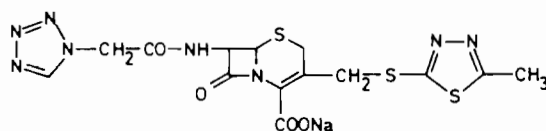
The polarographic behaviour of Cu(II) ions has been investigated in the presence of cefazolin (CEF). The data obtained from this study suggested that a complex of the form $\text{Cu}(\text{CEF})_2$ was formed in solution. When the complex was isolated and characterized using elemental analysis and IR, diffuse reflectance and ESR spectroscopy, the data suggested that the carboxylate group in cefazolin was involved in a bridging coordination mode, that the Cu(II) ion was present in the complex in a distorted octahedral or five coordination mode, and that the complex was polymeric in nature.

Because of the increasing use of antibiotics in both human and animal medicine, much attention has been paid in recent years to the interaction of such compounds with biometals [1–4], since complexation reactions occurring *in vivo* are of obvious pharmacological importance.

In this paper, we wish to report our findings on the complexation of cefazolin (CEF), 3-((5-methyl-1,3,4-thiadiazol-2-yl)thio)methyl)-8-oxo-7-((1H-tetrazol-1-ylacetyl)amino)-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylic acid, with copper(II) ions. Cefazolin is a semisynthetic cephalosporin derivative used parenterally and is usually formulated as the sodium salt which is highly soluble in aqueous solution [5].

The presence of several strong donor atoms and groups, in particular, the sulphur atoms and the tetrazole and thiadiazole rings, in the structure of cefazolin suggests that the molecule could act as a strong multisite ligand, especially with respect to transition metal ions. In an initial study on the complexation of cefazolin with Cu(II) ions, changes were observed in the reduction processes of Cu(II) ions in the presence of cefazolin, and in the main ultraviolet

absorption band of cefazolin [6]. No attempt was made at this stage, however, to characterize the complex formed, although the results suggested that the complex was of a 2:1 CEF:Cu(II) stoichiometry. It was decided, therefore, to isolate and characterize this complex and also to study the interaction in solution using differential pulse polarography (DPP).



Cefazolin, sodium salt (CEF)Na

Experimental

Materials

The sodium salt of cefazolin, (CEF)Na, was supplied in powder form and stored without further purification in a refrigerator. The stock solution of (CEF)Na for polarographic measurements was made freshly every 3 days by dissolution of the appropriate amount of (CEF)Na, calculated to anion form, in water and stored at *ca.* 4 °C. The stock solution of copper(II) ions was prepared by dissolution of an appropriate quantity of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in water. All reagents used were of analytical grade.

Preparation of the Complex

An aqueous solution of CuSO_4 (7.0 mg ml^{-1} Cu(II)) was added to cefazolin so that the molar ratio CEF:Cu(II) was 2:1. A green gelatinous precipitate and colourless solution immediately resulted. The mixture was stirred and left at room temperature for about 3 h. The voluminous substance was collected by filtration, thoroughly washed with water, and dried under vacuum over silica gel. Since the solid complex was not apparently soluble in water or in any common organic solvent, attempts at recrystallization were unsuccessful. Therefore, the dried complex was powdered, washed with a large amount of water and dried again as above. The final form of the complex was a light green powder.

Physical Measurements and Analyses

Polarographic measurements were recorded using a Solea-Tacussel polarograph (PRG 5) operated in conjunction with a polarographic stand CPR 3B. The electrode system was constructed with a platinum wire auxiliary electrode and a silver/silver chloride reference electrode together with the dropping mercury electrode as a working electrode. For all polarographic investigations, an 0.2 mol l^{-1} KNO_3 solution was used to maintain the ionic strength and a Britton–Robinson buffer (0.04 mol

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Γ^{-1} in boric acid, $0.04 \text{ mol } \Gamma^{-1}$ in phosphoric acid and $0.04 \text{ mol } \Gamma^{-1}$ in acetic acid with $0.2 \text{ mol } \Gamma^{-1}$ NaOH to adjust pH) was used for all measurements where a certain pH value was demanded. The solution in the polarographic cell was thermostatted at $25.0 \pm 0.2 \text{ }^\circ\text{C}$ before and during all measurements, and was deoxygenated by bubbling through with pure, oxygen-free argon.

Infrared spectra were recorded on a Perkin-Elmer Model 1330 infrared spectrophotometer in the region $4000\text{--}200 \text{ cm}^{-1}$. The spectra were obtained as KBr pellets and nujol mulls. The wavenumber accuracy was better than 4 cm^{-1} . Diffuse reflectance spectra of the powdered compound were obtained on a Perkin-Elmer Model 330 spectrometer. ESR X-band spectra were obtained on a Varian 4502 apparatus.

The content of copper and sodium was determined by atomic absorption spectroscopy (AAS) after prior decomposition of the complex with boiling nitric acid. Carbon, hydrogen, nitrogen and sulphur analyses were carried out by the Microanalytical Department at University College Dublin.

Elemental Analysis $\text{Cu}(\text{CEF})_2$

Anal. Found: C, 34.8; H, 2.8; N, 22.9; S, 19.2; Cu, 6.1. Calc: C, 34.7; H, 2.7; N, 23.1; S, 19.8; Cu, 6.6%.

Results and Discussion

Polarographic Behaviour

The polarographic behaviour and analysis of cephalosporin derivatives have been recently reviewed by Siegeman [7]. The reduction process for these compounds usually involves a $2e^-$ reduction of the $\text{C}=\text{C}$ double bond in the cephem nucleus. The reduction of carbon-carbon double bonds usually occurs at high negative potentials ($> -1.8 \text{ V}$), but is more easily facilitated in the case of cephalosporins by the presence of electron-withdrawing substituents.

When the differential pulse polarographic behaviour of Cu(II) ions was studied at the $5 \times 10^{-5} \text{ M}$ level in the presence of cefazolin in B.R. buffers of pH 2-7, the peak height due to the $2e^-$ reduction of free hydrated Cu(II) ions to Cu(0) was found to decrease in size on addition of increasing amounts of cefazolin (Fig. 1). In addition, a new peak appeared at a lower negative potential ($E_p(\text{I})$) due to the reduction of $\text{Cu}(\text{II}) \rightarrow \text{Cu}(\text{I})$ ions, whereas another peak appeared at a higher negative potential ($E_p(\text{II})$) due to the reduction of $\text{Cu}(\text{I}) \rightarrow \text{Cu}(\text{0})$ ions, and a further peak appeared at an even more negative potential due to the reduction of cefazolin, itself. The dependence of the E_p and i_p values for the reductions of $\text{Cu}(\text{II}) \rightarrow \text{Cu}(\text{0})$, $\text{Cu}(\text{II}) \rightarrow \text{Cu}(\text{I})$ and

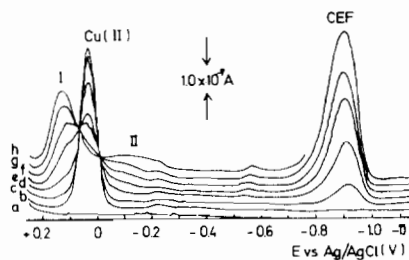


Fig. 1. DPP polarograms showing the additions of CEF to solution of $5 \times 10^{-5} \text{ mol } \Gamma^{-1}$ Cu(II). a: B.R. buffer + $0.20 \text{ mol } \Gamma^{-1}$ KNO_3 , pH = 4.8; b: a + $5 \times 10^{-5} \text{ mol } \Gamma^{-1}$ Cu(II); c: b + $1 \times 10^{-5} \text{ mol } \Gamma^{-1}$ CEF; d: b + $3 \times 10^{-5} \text{ mol } \Gamma^{-1}$ CEF; e: b + $5 \times 10^{-5} \text{ mol } \Gamma^{-1}$ CEF; f: b + $6 \times 10^{-5} \text{ mol } \Gamma^{-1}$ CEF; g: b + $8 \times 10^{-5} \text{ mol } \Gamma^{-1}$ CEF; h: b + $1 \times 10^{-4} \text{ mol } \Gamma^{-1}$ CEF. Experimental parameters: pulse amplitude 50 mV , drop time 0.9 s , scan rate $4 \text{ mV } \text{s}^{-1}$.

$\text{Cu}(\text{I}) \rightarrow \text{Cu}(\text{0})$ on increasing cefazolin concentration is shown in Fig. 2. The shifts observed in $E_p(\text{I})$ and $E_p(\text{II})$ are similar to those obtained using ligands such as ammonia, pyrazole and imidazole [8, 9].

When the relative DPP peak height for the $\text{Cu}(\text{II}) \rightarrow \text{Cu}(\text{0})$ reduction was plotted as a function of the molar ratio CEF:Cu (Fig. 3), it was found that all the Cu(II) ions were complexed when the ratio approached 2:1. In addition, it was shown that the complexation reaction was unaffected by pH in the range 2-7.

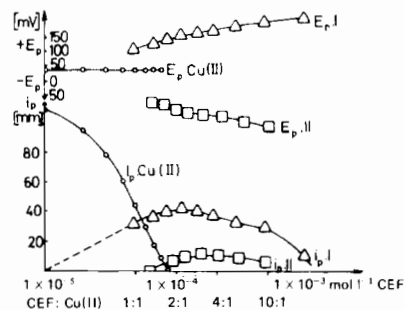


Fig. 2. Dependence of DPP peak heights and peak potentials of Cu(II), peak I and peak II on [CEF] at constant $[\text{Cu}(\text{II})] = 5 \times 10^{-5} \text{ mol } \Gamma^{-1}$. Medium: B.R. buffer + $0.2 \text{ mol } \Gamma^{-1}$ KNO_3 , pH = 4.2. Experimental parameters: as for Fig. 1.

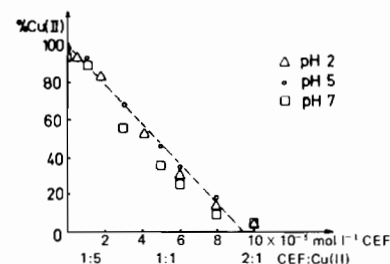


Fig. 3. Dependence of relative DPP peak heights of Cu(II) ions on pH and [CEF] at a constant $[\text{Cu}(\text{II})] = 5 \times 10^{-5} \text{ mol } \Gamma^{-1}$. Experimental parameters: as for Fig. 1.

Characterization of the Solid Complex

When electrochemical measurements were carried out at concentrations higher than 10^{-3} mol Γ^{-1} , a green precipitate was obtained. It was decided to further investigate the nature of this material, and a compound was prepared using the 2:1 ligand to metal ratio suggested by the electrochemical data. It was hoped that it would be possible to obtain information about the coordination mode of the ligand. It was anticipated that this would be difficult, however, as a large number of sites are available for coordination.

Elemental analysis showed that the material obtained corresponded well to the formula $\text{Cu}(\text{CEF})_2$. Analysis for sodium and sulphate proved negative. This suggests that the carboxylic acid groups attached to the cephem rings are deprotonated yielding a neutral complex. This is expected from a consideration of the pK_a value of 2.3 for cefazolin [5].

Infrared spectra of $\text{Cu}(\text{CEF})_2$, the sodium salt $\text{Na}(\text{CEF})$, and of the free acid were measured as nujol mulls and as KBr pellets. Although a complete assignment of the vibrational spectra is outside the scope of this work, a tentative assignment of the strong bands in the $\nu(\text{C}=\text{O})$ region has been given in Table I. The assignment is based on comparison with spectra of a group of related cephem-based antibiotics [10] and with literature values [11, 12]. Between 1800 and 1300 cm^{-1} $\nu(\text{C}=\text{O})$ vibrations of the lactam, the amide and of the carboxylic acid groups are expected. The peaks at 1780 and at 1680

cm^{-1} , have been tentatively assigned to the $\begin{array}{c} \diagup \\ \text{C}=\text{O} \\ \diagdown \end{array}$

stretching vibrations of the lactam and the amide group, respectively. Very little variation in these bands is found in the compounds studied (<5 cm^{-1}), so it seems likely that these groups are not coordinated to the metal ion [11].

The spectrum of the free acid was recorded to facilitate identification of the carbonyl stretching

frequencies of the carboxylic acid group. For both the COOH and COO^- groups, two carbonyl stretching vibrations are expected, but their peak positions are quite different [12]. The position of the stretching vibrations in COO^- -compounds depends both on the coordination mode of the COO^- grouping and on the bound metal ion. For the COO^- group, monodentate, bidentate and bridging coordination modes are possible, and it has been proposed that from the position of the carbonyl stretching vibrations an indication about the structure of the metal complexes can be obtained [11]. For the free acid, carbonyl stretching vibrations have been assigned to bands at 1720 and 1380 cm^{-1} . In the two salts, bands at about 1600 and 1400 cm^{-1} , have been assigned as the $\nu(\text{C}=\text{O})$ and $\nu(\text{C}-\text{O})$ vibrations respectively. These vibrations correspond to the $\nu_a(\text{COO}^-)$ and $\nu_s(\text{COO}^-)$ in the symmetric COO^- group. The fact that especially the $\nu(\text{C}=\text{O})$ band is metal dependent suggests that the carboxylic acid group is coordinated to the copper ion. The position of the bands and the magnitude of their separation seems to suggest a bridging coordination mode for the COO^- groups, although a monodentate coordination cannot be ruled out. The data suggest, however, that a bidentate coordination of the acid group is unlikely [11]. The vibrational spectra did not yield any information about a possible coordination of other active groups in the CEF molecule. The diffuse reflectance spectrum of the copper complex shows a broad asymmetric absorption with a low-energy shoulder at 1400 cm^{-1} . This suggests a distorted octahedral or five coordination for the central $\text{Cu}(\text{II})$ ion.

The ESR spectrum of the compound shows a single broad resonance (band width at half height 300 G) with a g-value of 2.10. This suggests the presence of strong Cu-Cu interactions. The ESR signal is consistent with a polymeric structure for the copper complex [13].

Conclusions

All data obtained support the formulation of $\text{Cu}(\text{CEF})_2$ for the green copper complex. From infrared data, a coordination of the carboxylic acid groups in a bridging mode seems likely. Nothing can be said with certainty about the coordination of other groups complexed to the metal ion. It is most likely, however, that both the tetrazole and the thiazazole rings are involved in this process. From the ESR spectrum the polymeric nature of the material can be concluded. It seems unlikely, however, that the complex in solution has the same structure: a monomeric structure being more likely with possibly bridging carboxylic acid groups causing the precipitation of the polymeric complex.

TABLE I. Carbonyl Stretching Frequencies of some CEF Compounds.

Nature of $\nu(\text{C}=\text{O})$	$\text{Cu}(\text{CEF})_2^a$	$(\text{CEF})\text{Na}$	$(\text{CEF})\text{H}$
lactam	1775s	1770s	1785vs
carboxylic acid			1720vs
amide	1685s	1678s	1685vs
carboxylic acid	1593ms	1610s	
carboxylic acid	1385vbr,s	1402vs	1370s

^a All spectra measured as KBr disks.

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