The Formation of a Cu(II)–Amikacin Complex and its Biological Activity and Uses. Indirect Estimation of Amikacin by Atomic Absorption Spectroscopy (AAS)

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Abstract

The [Cu(amikacin)$(H_2O)_3$] complex was prepared and characterized by elemental analysis, TGA and spectroscopic techniques (*viz.* IR, electronic and EPR spectra). The spectral results obtained indicate distorted octahedral geometry around the Cu(II) ion. The orbital reduction factors $K_\parallel$ and $K_\perp$ have been calculated. Amikacin can be estimated indirectly by atomic absorption spectroscopy using carbonate as an auxiliary ligand. The complex shows biological activity towards six organisms. Amikacin is biologically active towards *Sarcina lutea*, whereas the Cu(II)–amikacin complex is biologically inactive.

Introduction

Amikacin is a semisynthetic aminoglycoside produced by the strategic chemical alteration of kanamycin [1]. It has found worldwide use in the treatment of serious Gram-negative infections. Despite the importance of aminoglycosides and their metal chelates, little attention has been paid to them by previous authors [2, 3]. In addition, no systematic studies are present in the literature concerning complex formation between amikacin and the Cu(II) ion. The methods utilized for the analysis of amikacin include volumetric [4], colorimetric [5] and microbiological [6].

The aim of the present work was to synthesize the Cu(II)–amikacin complex and to characterize it by elemental analysis, TGA, IR, electronic and EPR spectra, in order to throw more light on its structure and geometry. This led to the development of an indirect method for the determination of amikacin by atomic absorption spectroscopy (AAS). Moreover, the biological utilization of the complex has been tested against seven organisms.

Experimental

All the chemicals used in the present investigation were Aldrich or BDH pure grade. Copper sulfate solution was standardized by the recommended methods [7]. A stock solution of amikacin was prepared by dissolving a definite weight of amikacin sulfate in the appropriate volume of water.

Complex Preparation

A solution of 5.0 g KOH dissolved in 10 ml H_2O was mixed with 0.03 mol amikacin in 25 ml H_2O then diluted to 50 ml with H_2O and mixed with 50 ml ethanol. The mixture was stirred to dissolve the ligand. A solution of 0.03 mol Cu(II) sulfate (7.5 g dissolved in 20 ml of distilled H_2O) was added dropwise, whereby a blue solid immediately precipitated. The separated solid was filtered off, washed with ethanol and ether then dried *in vacuo*.

The working procedure and apparatus utilized for investigating the solid complex were the same as given before [8, 9].

The Atomic Absorption Determination of Amikacin

To a solution of amikacin sulfate, 1 ml of $CuSO_4$ (1.5%) and 1 ml of Na_2CO_3 (1.5%) were added, diluted to 10 ml, then the mixture was centrifuged. The atomic absorption spectrophotometer was used to determine the copper concentration in the supernatant. The concentration of amikacin was calculated by reference to a calibration curve. The present method has a low value of standard deviation (1.28), calculated for 10 measurements, Table I.

The apparatus used was a Beckman DB grating spectrophotometer equipped with a Beckman

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TABLE I. Standard Deviation for 10 Measurements of Indirect Estimation of Amikacin by Atomic Absorption Spectroscopy (AAS)

Experiment No.	Y_{Am} Taken (γ/ml)	Y_{Am} Found (γ/ml)	% Recovery (x)[a]	$(\bar{x} - x)$	$(\bar{x} - x)^2$
1	5.0	4.92	98.4	0.911	0.828
2	10.0	9.71	97.1	2.211	4.888
3	15.0	14.76	98.4	0.911	0.829
4	20.0	20.29	101.45	−2.139	4.575
5	25.0	24.61	98.42	0.887	0.786
6	30.0	28.91	96.36	2.951	8.708
7	40.0	40.59	101.48	−2.164	4.682
8	50.0	50.44	100.88	−1.569	2.461
9	60.0	60.28	100.46	−1.149	1.320
10	70.0	70.12	100.17	−0.859	0.737

[a]Average value of $x = 99.311 \pm 1.28$.

potentiometric recorder and a Beckman laminar flow burner. The copper hollow lamp was neon and equipped by Beckman. The flame was used with the following settings: wave length, 324.7 nm; lamp current, 10 mA; slit width, 0.2 mm; air pressure, 20 psi; acetylene pressure, 20 psi; burner height, 2.5 inch.

Microbiological Measurements

The assay tests were carried out according to a diffusion method [10]. The data obtained are recorded in Table II.

TABLE II. Antimicrobiological Potential of Amikacin and its Cu(II) Complex

Microorganism	Amikacin	[Cu(amikacin)$(H_2O)_3$]
Saccharomyces cervisae (I)	50	40
Bacillus cereus (II)	40	40
Klebsiella aerogenes (III)	40	45
Staphylococcus aureus (IV)	45	45
Sarcina lutea (V)	40	
Escherichia coli (VI)	26	30
Candida albicans (VII)	43	45

Results and Discussion

On the basis of the analytical data obtained, the Cu(II)–amikacin complex can be formulated as [Cu(amikacin)$(H_2O)_3$]. The molar conductance of the prepared complex in DMF is less than 8.0 ohm^{-1} cm^2 mol^{-1}, indicating that the complex is a non-electrolyte [11] and hence amikacin would behave as a dibasic acid on reaction with Cu(II) ion. This confirms that the Cu(II) ion forms with amikacin a 1:1 complex. The complex contains three water molecules coordinated to the central Cu(II) ion. This conclusion is also supported by the results of thermogravimetric analysis of the Cu(II)–amikacin complex. The upper part of the thermograms show the dehydration of water molecules from the complex under investigation at 140–150 °C. The loss in weight amounts to 7.70% (found, 7.60%), which corresponds to three water molecules in the complex.

The existence of water of coordination renders it difficult to draw conclusions regarding the ν_{OH} band since it is covered by those of the water molecules. The spectra of the Cu(II)–amikacin complex exhibit a broad band at 3520–3480 cm^{-1} corresponding to ν_{OH} of coordinated water. The presence of the new band in the region 805–800 cm^{-1}, assigned to the out-of-plane deformation vibrations of coordinated water [12, 13], supports the presence of coordinated water molecules.

The IR spectrum of Cu(II)–amikacin complex displays interesting changes in comparison to that of the free ligand. (i) The bands observed at 3350 and 3220 cm^{-1} in the spectra of free amikacin due to ν_{OH} and ν_{NH}, respectively, are shifted by 10–15 cm^{-1} to lower wavenumbers in the spectra of the Cu(II)–amikacin complex but are of lower intensity; this suggests that one NH_2 and one OH alcoholic group contribute to chelation. (ii) The ν_{CONH} of the free amikacin, located at 1640 and 1580 cm^{-1}, tends to vanish and instead a new one appears at 1610 cm^{-1} which can be assigned to $\nu_{C=N}$ as a result of the ligand enolization (–CONH– to –C(HO)=N–) on complex formation. The non-appearance of the enolic OH group in the spectrum of the complex denotes the participation of the enolic form in chelation through H^+ displacement. This is in accordance with the results of conductance measurements which

indicate that amikacin behaves as a dianionic species on reaction with Cu(II) ion. (iii) The two new bands observed at 380 and 475 cm^{-1} in the spectrum of the Cu(II) complex can be assigned to ν_{M-N} and ν_{M-O} respectively.

The electronic absorption spectrum of the Cu(II)–amikacin complex comprises two broad bands with λ_{max} at 395 and 625 nm that can be assigned to the electronic transitions: $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2E_g$, respectively, within the energy level diagram for the Cu(II) ion in a strong tetragonally elongated octahedral symmetry.

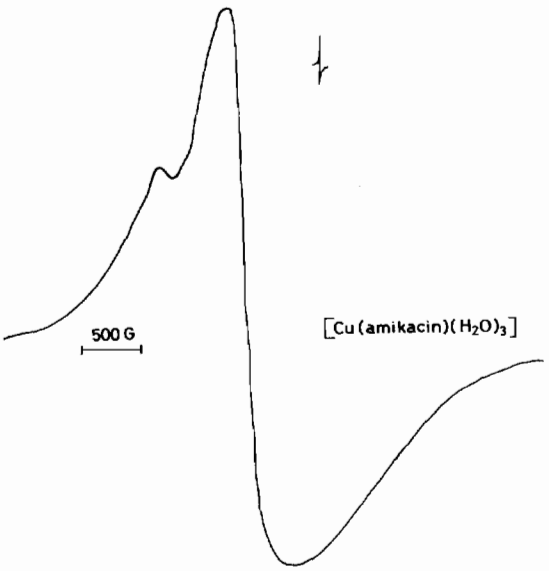


Fig. 1. The X-band EPR spectrum of the Cu(II)–amikacin complex at room temperature.

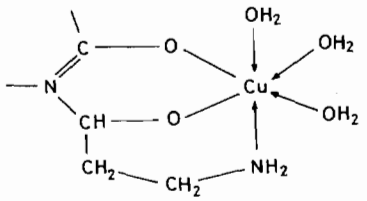
The X-band EPR spectrum of the Cu(II)–amikacin complex measured at room temperature exhibits an intense broad band with two lines at $g_\parallel = 3.2322$ and $g_\perp = 2.5139$ with no obvious hyperfine structure (Fig. 1). The calculated value of g_{iso} [$g_{iso} = \frac{1}{3}(2g_\perp + g_\parallel)$] for the Cu(II)–amikacin complex amounts to 2.7534, which is in reasonable agreement with the observed values for g_{iso} of Cu(II) in similar axial symmetry [14]. The values of $G = (g_\parallel - 2)/(g_\perp - 2) < 4.0$, suggesting the presence of significant exchange coupling and that the local tetragonal axes are misaligned [15, 16]. According to ligand field theory [17], g-values of copper(II) complexes of axial symmetry may be approximated by the equations:

$$g_\parallel = 2\left(1 - \frac{4\lambda K_\parallel}{\Delta E(d_{x^2-y^2} - d_{xy})}\right) \quad (1)$$

$$g_\perp = 2\left(1 - \frac{\lambda K_\perp}{\Delta E(d_{x^2-y^2} - d_{xz})}\right) \quad (2)$$

where λ is the spin–orbit coupling constant of the copper ion and $K_\parallel$ and $K_\perp$ are the orbital reduction factors for the $\parallel$ and $\perp$ components, respectively. By using relations (1) and (2) and the values of the spectroscopically obtained $\Delta E(d_{x^2-y^2} - d_{xz})$ and $\Delta E(d_{x^2-y^2} - d_{xz})$ values, $K_\parallel = 0.5009$ and $K_\perp = 0.5272$, respectively.

Based on the above knowledge, the bonding between the Cu(II) ion and amikacin can be represented by the following structure:



Indirect Determination of Amikacin by AAS

Amikacin was simply allowed to react with the Cu(II) ions present in solution at pH 10 in carbonate medium, where the respective copper complex was formed. This complex was directly aspirated and atomized in the instrument; the absorbance recorded was due to the copper that reacted with amikacin, while the excess of copper was precipitated as the carbonate. Thus, the amikacin content can be easily calculated with the aid of pre-drawn calibration curves of either copper sulfate or copper amikacin (Fig. 2). To calculate the amikacin content, one can use the following equation:

$$Y_{Am} = \frac{XVW_{Am}}{W_{Cu}} = 9.222xv$$

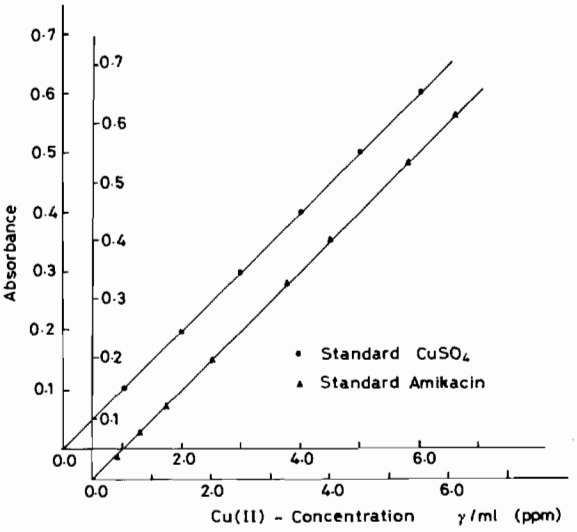


Fig. 2. Calibration curves for standard copper sulfate (•) and standard amikacin (▲).

where: Y_{Am} = concentration of amikacin, X = concentration of Cu(II) ion, V = total volume, W_{Am} = molecular weight of amikacin and W_{Cu} = molecular weight of Cu.

It has been found that Beer's law is obeyed in the range 0.05 to 5.0 μg of amikacin initially present in solution.

Biological Activity of the Cu(II)–Amikacin Complex

The results gained from testing amikacin and the Cu(II)–amikacin complex against seven microorganisms (Table II) reveal the following:

(a) The complex has the same biological activity towards **(II)** and **(IV)** as amikacin itself.

(b) The complex shows higher activity towards **(III)**, **(VI)** and **(VII)** than amikacin itself.

(c) Amikacin is biologically active towards **(V)**, while the complex is biologically inactive.

(d) The biological activity of the complex can be arranged in the following order: **(III)** ~ **(IV)** ~ **(VII)** > **(I)** ~ **(II)** > **(VI)**; whereas the biological activity of amikacin can be arranged in the following order: **(I)** > **(IV)** > **(VII)** ~ **(II)** ~ **(III)** ~ **(V)** > **(VI)**.

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