

# Spectrophotometric studies of copper-streptomycin complex and determination of streptomycin sulphate using an auxiliary ligand

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## Abstract

Spectrophotometric investigation of the deep blue, water soluble complex of streptomycin sulphate with bivalent copper in the alkaline medium has been carried out in presence of an auxiliary ligand sodium potassium tartrate. The complex (at pH 8.5) is found to absorb at 650 nm. Job's method and the mole ratio method have shown the composition of the complex to be 1 : 1. Conditional stability constant has been determined by Mukherjee and Dey method and Raghav Rao's method. The log  $K_c$  value by these two methods is found to be 3.98 and 3.93 respectively.

The complex obeys Beer's law in the range of 0.25 to 5 mg streptomycin sulphate/ml of the solution. The technique is found to be suitable for the quantitative estimation of streptomycin.

**Key words:** Auxiliary ligand, Copper, Streptomycin, Tartrate-sodium potassium, Complex-Copper-Streptomycin, Stability constant, Spectrophotometry.

## 1. Introduction

Streptomycin, a well known member of aminoglycoside antibiotic group, has been studied for its complex forming properties by Foye *et al.*<sup>4</sup> On the basis of quantitative analysis, they reported 3 : 1 interaction between copper and streptomycin sulphate. On the other hand, Zhan and Eisenbrandt<sup>10</sup> found the composition to be 1 : 1 by potentiometric method. The stability constant data of the complex were determined<sup>1</sup> by pH titration.

Sodium potassium tartrate has been found to be effective as an auxiliary ligand in the spectrophotometric investigation of other aminoglycoside antibiotics neomycin and kanamycin.<sup>3</sup> With a view to resolving the controversy regarding the composition of copper(II)—streptomycin complex and to explore the possibility of spectrophotometric determination of streptomycin sulphate, the present investigation was taken up in presence of the auxiliary ligand.

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## 2. Experimental

Spectrophotometric measurements were done with a Beckman spectrophotometer model DU-2400. A Beckman pH meter with a glass and calomel electrode assembly was used for all pH measurements.

All the reagents used were of analytical grade. A sample of I.P. grade streptomycin sulphate (SMS) was supplied by Alembic Chemical Works Ltd., Baroda. Fresh solution of copper sulphate was standardised iodometrically and diluted to the desired concentration. Copper tartrate (CuT) solution was prepared by adding four times excess of sodium potassium tartrate to copper sulphate solution and diluting it to a definite volume to give a standard solution with respect to copper(II) content.

All experiments were carried out at  $30 \pm 1^\circ \text{C}$ . The pH of the solution was adjusted by the addition of a dilute solution of HCl or NaOH within the limits of  $\pm 0.1$ .

*Absorption spectra and the effect of pH*: A series of solutions, each containing  $5 \times 10^{-3} \text{ M CuSO}_4$  and two-fold excess of SMS, was prepared with different pH values ranging from 6.0 to 9.0. Their optical densities were measured in the visible range from 500 nm to 740 nm. The maximum colour intensities were obtained within the pH range 8.0 to 8.5 with  $\lambda_{\text{max}}$  at 650 nm.

*Absorption spectra of Cu-SMS and CuT complexes*: Hydrolysis of copper in the alkaline medium presented difficulty in the spectrophotometric investigation of Cu-SMS complex. This difficulty was, however, overcome by using sodium potassium tartrate as an auxiliary ligand which forms a 1:1 blue coloured complex with copper in the alkaline medium.<sup>7</sup>

The absorption spectra of Cu-SMS and CuT complexes were compared by preparing the following set of solutions and recording their optical densities at pH 8.5 from 500 nm to 740 nm (Fig. 1).

- (A)  $5 \times 10^{-3} \text{ M CuSO}_4$  solution containing four-fold excess of sodium potassium tartrate.
- (B) Solution A to which  $10 \times 10^{-3} \text{ M}$  solution of SMS was added.
- (C)  $5 \times 10^{-3} \text{ M CuSO}_4$  to which two-fold excess of SMS was added.

*Stoichiometry of the complex*: Job's method of continuous variation<sup>5</sup> with equimolar and non-equimolar solutions of the reagents and mole ratio<sup>9</sup> method were employed for determining the composition of the complex. A number of series of solutions were prepared, each series with a different concentration of CuT and SMS solutions, in the usual manner and their optical densities at 600 nm was recorded. Optical densities were also recorded of the same number of CuT solutions, used as a blank, which was

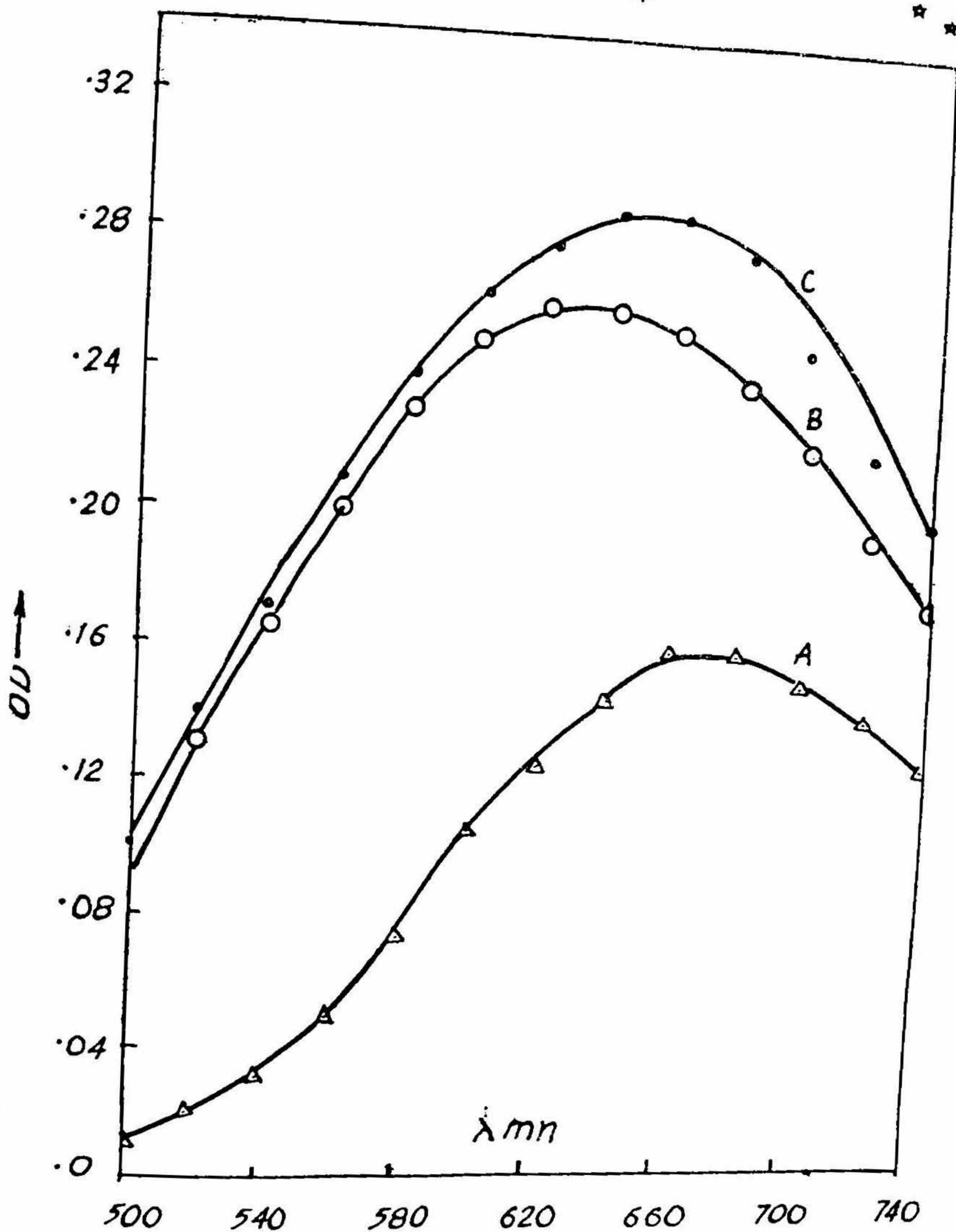


FIG. 1. Absorption spectra of CuT and Cu-SMS complexes.

Composition of the solutions:

Curve A.  $5 \times 10^{-3}$  M  $\text{CuSO}_4$  +  $20 \times 10^{-3}$  M sodium potassium tartrate.

Curve B. Composition of A +  $10 \times 10^{-3}$  M SMS.

Curve C.  $5 \times 10^{-3}$   $\text{CuSO}_4$  +  $10 \times 10^{-3}$  M SMS.

prepared under identical conditions except the addition of SMS. The difference in the optical densities ( $\Delta O \cdot D.$ ) of each of the above solutions and the corresponding blank was plotted against the mole fraction of CuT to obtain Job curves and against the molar ratio of the reagents to obtain molar ratio curves.

### 3. Results and discussion

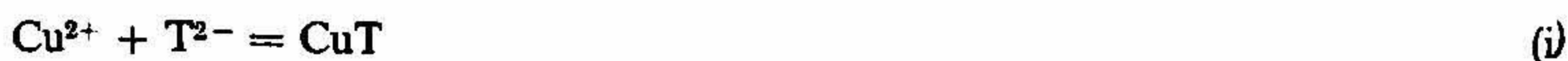
A comparison of the curves A and C (Fig. 1) clearly shows that the absorption by CuT is much less than that by an equimolar solution of Cu-SMS complex. Moreover, the fact that curve B is similar in structure to curve C, suggests the quantitative conversion of CuT complex forming Cu-SMS complex on addition of SMS. It was therefore inferred that sodium potassium tartrate could be used as an auxiliary ligand for the spectrophotometric investigation of Cu-SMS complex.

The maximum difference in the optical densities of CuT and Cu-SMS complexes at 8.5 pH was observed at 600 nm and so these conditions were selected for all further studies. Any increase in the optical densities of CuT complex solution, at this wavelength, upon adding SMS solution was regarded as a measure of complexation between copper and streptomycin.

All the Job curves show a maximum corresponding to the formation of 1:1 complex between copper and streptomycin. The straight portions of the molar ratio curves, upon extrapolation, intersected at points which also gave the stoichiometric ratio of 1:1. This inference is in agreement with the potentiometric studies.

*Evaluation of the stability constant of the complex:* The molar value of stoichiometric constants are reliable under a given set of experimental conditions and are useful for practical purposes. In the present study the constants determined are those obtained at fixed temperature and pH as mentioned and in presence of sodium potassium tartrate. This constant has been termed conditional stability constant in the present work.

Formation of CuT complex in the alkaline medium and its equilibrium constant can be represented by the following equations.



$$K_1 = (\text{CuT})/(\text{Cu}^{2+})(\text{T}^{2-}). \quad (\text{ii})$$

Replacement of tartrate ion by SMS and the corresponding equilibrium constant can be represented by equations (iii) and (iv) respectively.



$$K_2 = (\text{CuSMS}^{2+})(\text{T}^{2-})/(\text{CuT})(\text{SMS}), \quad (\text{iv})$$

By comparing equations (ii) and (iv) it can be shown that the conditional stability constant  $K_s$  of Cu-SMS complex can be written as,

$$K_s = \frac{(\text{Cu SMS}^{2+})}{(\text{Cu}^{2+})(\text{SMS})} = K_1 K_2.$$

The value of  $1/K_1$  was reported<sup>7</sup> to be  $8.15 \times 10^{-2}$  while  $K_2$  was determined by two different methods, *i.e.*, the method of Mukherjee and Dey<sup>6</sup> and the Raghav Rao's method.<sup>8</sup> The free energy of formation of the complex has also been calculated with the help of the following expressions:

$$\Delta F = -RT \ln K_s.$$

The terms have their usual meanings. The results of the conditional stability constant and free energy of formation of the complex are shown in Table I.

Table I

*Stability constant and free energy of formation of Cu-SMS complex at  $30 \pm 1^\circ \text{C}$*

Method	$\log K_s$	$\Delta F$ K · cal/mole	Reference
Mukherjee and Dey	3.98	-5.58	..
Raghav Rao	3.93	-5.51	..
Bjerrum	3.72	..	1

#### *Colorimetric determination of streptomycin sulphate*

*Beer's Law* : Varying quantities (.1, .2, .3, ... 1.0 ml) of  $4 \times 10^{-2}$  M SMS solution was added to an excess of the CuT complex solution and the volume was made up to 4 ml while adjusting the pH to 8.5. The colour intensities were measured at 600 nm against a CuT blank. It was found that the system adheres to Beer's law within the range 0.25 mg to 5 mg streptomycin sulphate/ml of the solution.

Thus this technique provides a new and quick method for the determination of streptomycin sulphate in the pharmaceutical preparations. As the method is based upon the decomposition of Cu-Tartrate complex, any impurity which may form complex with copper will interfere.

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