

Research Article

Spectrophotometric Determination of Copper by using Erythromycin

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Abstract

Copper (II) forms a complex with erythromycin at pH 5 which leads to spectroscopic determination of erythromycin using (UV-Vis). Erythromycin-Copper (II) complex was stabilized and measured at 404 nm while Beer's law is valid for the concentration range of 2.5-30 $\mu\text{g}\cdot\text{ml}^{-1}$. Sandell's sensitivity was 0.284738042 $\mu\text{g}\cdot\text{cm}^{-1}$, However the detection limit was 0.274576 $\mu\text{g}\cdot\text{ml}^{-1}$, and the limit of quantitation was 0.915255 $\mu\text{g}\cdot\text{ml}^{-1}$. Mole ratio method was applied with result (1:1 L:M) Also Continuous Variation Method was applied.

Keywords: Antibiotic drug; Erythromycin; Erythromycin complex; Determination of copper

Introduction

Erythromycin is very active against Gram-positive and some Gram-negative microorganisms which is used for treatment of respiratory, gastrointestinal, and genital tract infections Figure 1. This work develops an easy method and environmental friendly by using Metal Complexation. A complex between a erythromycin and copper ion is used as a chemo sensing that the complexation leads to a change in colour or fluorescence [1-3]. Transition metal complexes often have spectacular colours caused by electronic transitions by the absorption of light [4,5]. Each wavelength of light has a particular energy associated with it and then the solution of the mixture show the colour of the light it absorbs A charge transfer band entails promotion of electrons from a metal-based orbital into an empty ligand-based orbital (Metal-to-Ligand Charge Transfer or MLCT). UV-Vis spectrometry for its simplicity, versatility, speed and cost-effectiveness. UV-Vis spectrophotometers for environmental monitoring receives much attention once again. The metal ion recognition system is based on the interaction/complexation of the various ligands with heavy metals to form highly coloured complexes. There is a wide choice in commercially available chromo-reagents for heavy metal ion sensing [6,7]. Heavy metals can form a strong coloured complex. The molar absorptivity (ϵ , from Beer-Lambert's Law), is the parameter to evaluate how strong the heavy metals-indicator complex absorbing light at the given wavelength is. It is the intrinsic property of the absorbing species [8,9]. In this work exploring a method for determination of Copper by using erythromycin.

Apparatus

- UV-Visible recording spectrophotometer Shimadzu Model (160A) with a response time of 0.1s. A quartz cell of 5 ml internal volume and 1cm path length was used for absorbance measurements
- Hotplate Stirrer (Hotplate stirrer Model L-81Labinco bv)
- PH-meter (model BP 3001).

Materials

- A pure grade of erythromycin was obtained from Egyptian International Pharmaceutical Industries Company (EIPICO)
- Stocks solution were prepared from analytical grade BDH
- Preparation of Standard Solutions: All glassware used was cleaned with distilled water and dried at 50°C for 30 minute prior to use. Batch experiments were carried out in to ensure the reproducibility of results and the average value. Metal used were of the highest purity and most solutions were prepared in distilled water.
- 250 μgml^{-1} Stock solution of erythromycin prepared by dissolving 0.025g from erythromycin ($\text{C}_{37}\text{H}_{67}\text{NO}_{13}$) in distilled water and diluting to the mark in 100ml volumetric flask.
- A solution of 500 ppm of Cu^{+2} was prepared by dissolv-

ing 2.1557 gm of CuCl_2 in small amount of Water and complete the volume to 1000 ml by using volumetric flask.

Interference Solutions of 1000 ppm

1000 $\mu\text{g ml}^{-1}$ stock solution of interferences is prepared by dissolving 0.1g of the different organic compound such as [Lactose, Starch, Arabic Gum, Glucose and Talc] and inorganic compound such as of $\text{Ca}_3(\text{PO}_4)_2$ and CaCO_3 by 0.2579g and 0.2500g respectively in distilled water and diluting them to the mark in 100 ml volumetric flask.

Results and Discussion

Absorption Spectra

The absorption spectrum of the copper complex product formed was also recorded against the corresponding metal blank between 220 to 900 nm before obtaining optimum conditions show an absorbance at a wave length at 404nm. The molar absorptivity value is $3.4308 \times 10^{-4} \text{ L mol}^{-1} \text{ cm}^{-1}$. The value of molar absorptivity enables to carry out the quantitative analysis of Erythromycin .

Effect of pH: The maximum sensitivity was obtained at pH 5 to find the best acidic function at different value of pH 1-14 The results are shown in Figure 2, at pH =5 for Cu(II) show the value of absorbance intensity for the complexes ERY- Cu against the value of pH , the best values of pH recorded for the highest absorbance values were Plotting of the absorbance values versus the value of pH is shown in Figure 2.

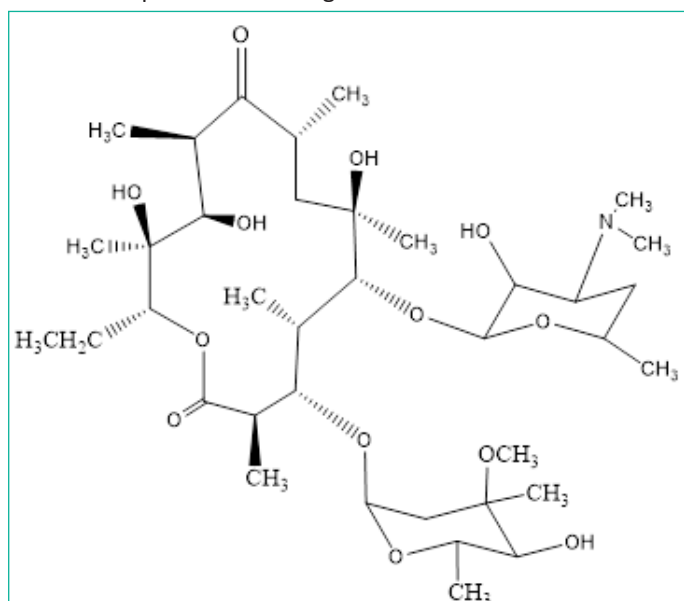


Figure 1: Erythromycin structure.

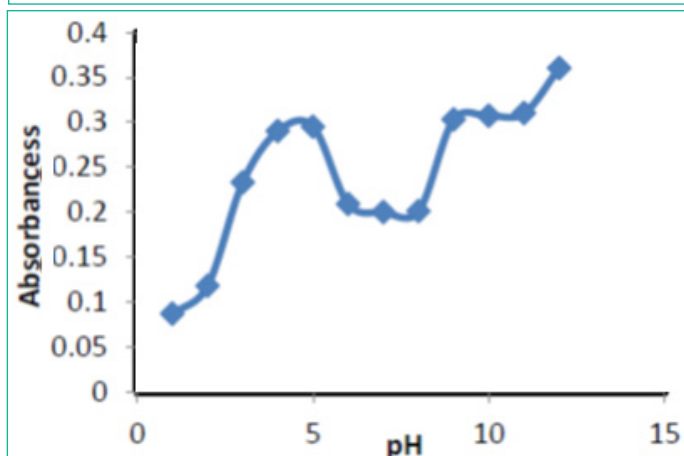


Figure 2: pH effect on absorbance of (Ery-Cu II) complex.

Effect of buffer solutions: The best values of buffer pH 5 recorded for the highest absorbance values were, The absorbance is measured the absorbance results are shown in Table 1. for complexes (Cu- Ery)

Effect of Volumes buffer solutions: Figure 3 shows the value of absorbance intensity for the complexes Ery- Cu against the value of buffer solutions , the best value of Potassium chloride buffer solutions recorded for the highest absorbance values

It is evident that absorbance increases with increase the volume of buffer, but suddenly decrease the absorbance because the decomposition happen when increase basicity.

Effect of the Equilibration Temperature and Time

To optimize this method, it was necessary to examine the effect of the temperature on complex. Temperature that enhances higher range of (35 – 90) $^{\circ}\text{C}$ and (5 - 50)min, respectively, while keeping all other parameters constant. Excellent absorbance was found at temperature 75 $^{\circ}\text{C}$ as shown in Figure 4; therefore choose 75 $^{\circ}\text{C}$ higher than is probably due to the decomposition of the complex.

Time was investigated in the range of (5-50) min .Excellent absorbance found at 35min and was selected to fulfill efficient separation conditions Figure 5.

Effect of Interference: The effect of some foreign compounds, which may found in environmental , were studied by adding 1ml of (100 $\mu\text{g ml}^{-1}$) Equal amounts organic compounds, Inorganic compounds to 1ml of (100 $\mu\text{g ml}^{-1}$) of complex . The color was developed following the recommended procedure described earlier Table 2.

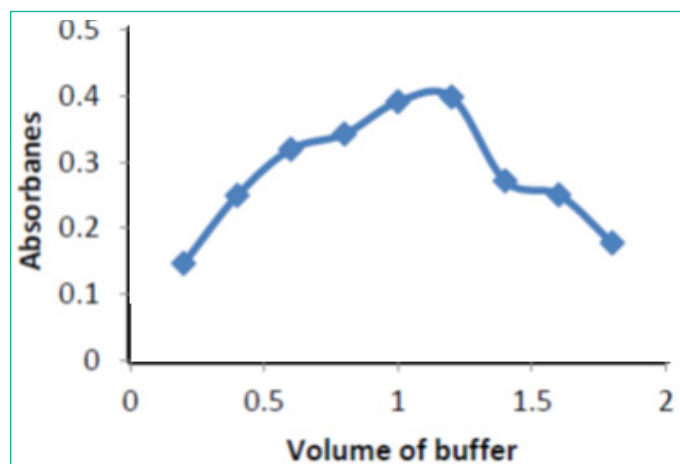


Figure 3: Buffer of pH effect on absorbance of (Ery-Cu II) complex

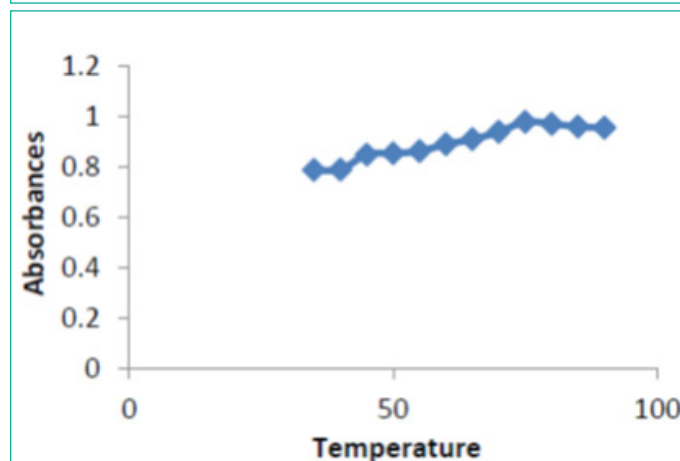


Figure 4: Effect of temperature on absorbance of (ERY-CU II) complex.

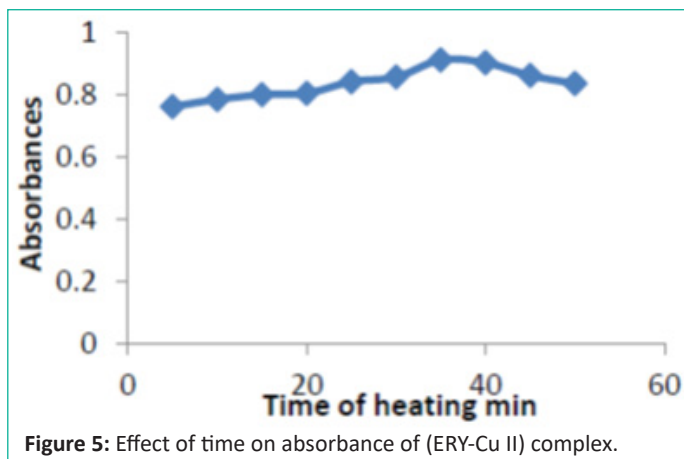


Figure 5: Effect of time on absorbance of (ERY-Cu II) complex.

Table 1: effect of buffers pH 5 on absorbance.

| Preparation buffer pH 5 | Absorbance |
|---------------------------------|------------|
| Sodium citrate | 0.052 |
| Sodium acetate buffer solutions | 0.392 |

Table 2: Effect of Interference.

| 100ppm interference | Absorbance at $\lambda_{max}=404$ for Cu |
|---|--|
| With out | 0.982 |
| Lactose | 0.056 |
| Starch | 0.232 |
| Arabic Gum | 0.094 |
| Talc | 0.046 |
| Glucose | 0.0001 |
| Ca ₃ (PO ₄) ₂ | 0.146 |
| CaCO ₃ | 0.167 |

Table 3: The optimum conditions for the determination of ERY.

| Optimum | Concentrations | Range selected | Optimum quantities of complex (ery-cu) |
|--|----------------|----------------|---|
| λ max(nm) | ---- | 220-1100 | 404 |
| Effect of volume of metal ion required | 500 ppm | 0.05-0.55ml | 0.4 |
| Effect of PH | 0.1M(Na OH) | 14-Jan | 12 |
| Buffer pH | | ---- | |
| Effect of volume of Buffer | ---- | 0.2-1.8ml | 1.2 |
| Effect of time heat-ing | ---- | 5-50min | 35min |
| ery solution re-quired | 250 ppm | 0.1-1.2ml | 0.5 |

Table 4: The absorbance measurements of standard solutions of complex (Ery-Cu).

| Recovery% | Found | RSD% | Mean Absorbance | Conc. ppm |
|-----------|---------|----------|-----------------|-----------|
| 89 | 2.2478 | 0.151903 | 0.465 | 2.5 |
| 96 | 4.8326 | 0.240513 | 0.587 | 5 |
| 95 | 7.1843 | 0.101087 | 0.698 | 7.5 |
| 99 | 9.9597 | 0.085245 | 0.829 | 10 |
| 105 | 13.1377 | 0.288615 | 0.979 | 12.5 |
| 101 | 15.2563 | 0.065503 | 1.079 | 15 |
| 101 | 17.8411 | 0.058852 | 1.201 | 17.5 |
| 101 | 20.2563 | 0.214926 | 1.315 | 20 |
| 100 | 22.5021 | 0.248719 | 1.421 | 22.5 |
| 99 | 24.875 | 0.092191 | 1.533 | 25 |
| 99 | 27.2478 | 0.042998 | 1.645 | 27.5 |
| 99 | 29.8326 | 0.199917 | 1.767 | 30 |

Indicating interfering with the determination at levels found in complex form.

Selected Optimum Conditions: The optimum conditions for the proposed procedure were summarized in Table 3 and were used in all subsequent experiments.

Calibration Graph

By applying the optimum conditions which described in the procedure liner calibration graph of Ery with Copper was obtained Figure 7, which show Beer law obeyed over the concentration range of 2.5-30 $\mu\text{g ml}^{-1}$ with correlation coefficient equal to 0.9989. All other analytical characteristics data are summarized in Table 4.

Optical Characteristics Features of the Calibration Curve

Stoichiometric Determination of Color complex:

Mole – Ratio Method

Aliquots of 10 mL solution containing (1×10^{-4}) mol L⁻¹ of (1mL) Erythromycin and increasing concentrations (1×10^{-4}) mol L⁻¹ of (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2) mL of (Cu) Copper (2×10^{-6} – 2×10^{-5}) mol L⁻¹ metal. The absorbance of the solutions were measured by UV-Vis spectrophotometer versus blank at λ max= 404 nm the stoichiometric ratio between 1:1 results are shown in the Table 6.

Continuous Variation Method (Job`s method)

A series of (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9) ml of (1×10^{-4}) mol L⁻¹ of the solution that contain erythromycin was pipette into each of 10 ml volumetric flask then (0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1) ml of (1×10^{-4}) mol L⁻¹ of metal the absorbance of the solution was measured by UV-Vis Spectrophotometer at λ max 404 nm the stoichiometric ratio between erythromycin with metal 1:1 results are shown in the Table 7 Plotting the value of absorbance versus the VD / VT is shown in Figure 9.

Table 5: shows the main features of the calibration curve and measuring the absorbance at 404 nm.

| Parameter | Complex (ery-cu) |
|--|------------------------------------|
| Wave length λ max (nm) | 404nm |
| Concentration rang ($\mu\text{g ml}^{-1}$) | 2.5-30 $\mu\text{g ml}^{-1}$ |
| Regression equation | $y = 0.0472x + 0.3589$ |
| Correlation coefficient(r) | 0.9994 |
| Correlation coefficient (r2) | 0.9989 |
| Variation coefficient (%) | 99.89 |
| Limit of Detection ($\mu\text{g ml}^{-1}$) | 0.274577 $\mu\text{g ml}^{-1}$ |
| Limit of Quantitation ($\mu\text{g ml}^{-1}$) | 0.915255 $\mu\text{g ml}^{-1}$ |
| Sandell's sensitivity ($\mu\text{g cm}^{-2}$) | 0.284738042 |
| Slope (m) | 0.0472 |
| Intercept (C) | 0.3589 |
| Molar absorptivity(L.mol ⁻¹ .cm ⁻¹) | 2.5×10^3 |
| Composition of product | 0.042361111 |
| C.L for slope (b \pm tSb) at 95 % | $0.0472 \pm 1.0858 \times 10^{-3}$ |
| C.L for intercept (a \pm tSa) at 95 % | 0.3589 ± 1.93298 |
| C.L for Conc. 5 $\mu\text{g ml}^{-1}$ at 95% | $0.587 \pm 4.9653 \times 10^{-3}$ |
| C.L for Conc. 15 $\mu\text{g ml}^{-1}$ at 95% | $1.079 \pm 2.4826 \times 10^{-3}$ |
| C.L for Conc. 25 $\mu\text{g ml}^{-1}$ at 95% | $1.533 \pm 4.9653 \times 10^{-3}$ |

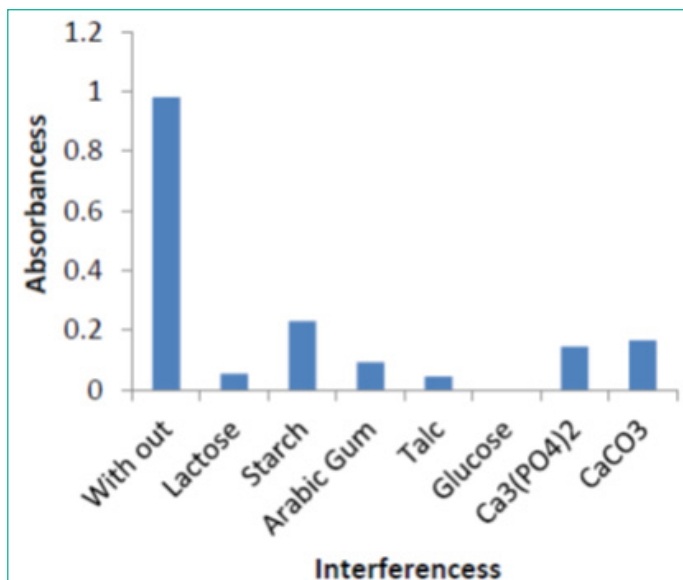


Figure 6: Effect of organic and inorganic Interferences on absorbance of (ERY-Cu II) complex.

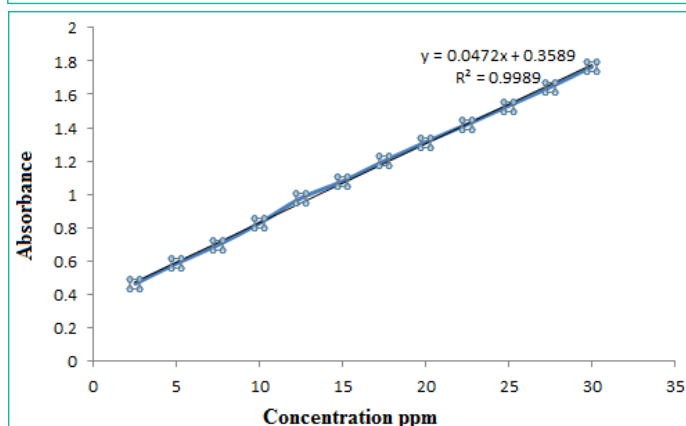


Figure 7: The calibration curve of (ERY-CU).

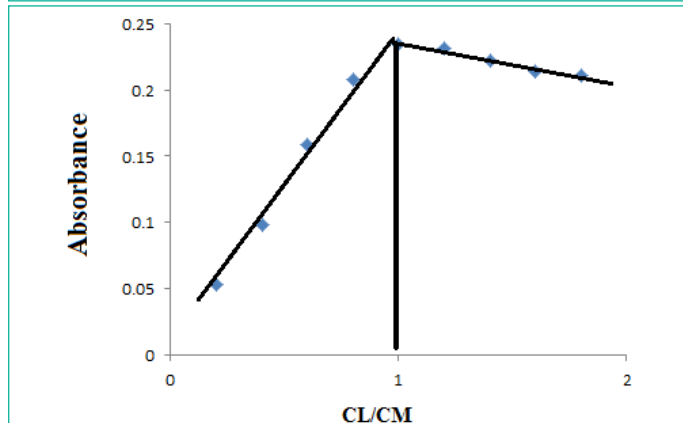


Figure 8: Molar ratio of Ery-copper complex.

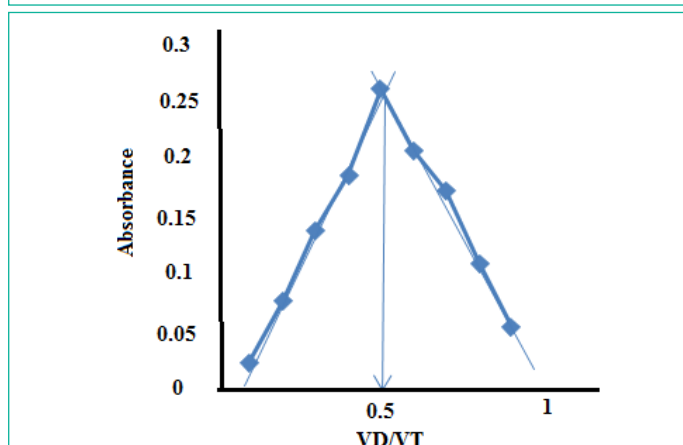


Figure 9: Continuous variation method plot.

Table 6: molar ratio of ERY-Cu.

| Absorbance | CL | CL/CM |
|------------|----------------------|-------|
| 0.053 | 2×10^{-6} | 0.2 |
| 0.098 | 4×10^{-6} | 0.4 |
| 0.159 | 6×10^{-6} | 0.6 |
| 0.208 | 8×10^{-6} | 0.8 |
| 0.234 | 1×10^{-5} | 1.0 |
| 0.231 | 1.2×10^{-5} | 1.2 |
| 0.222 | 1.4×10^{-5} | 1.4 |
| 0.214 | 1.6×10^{-5} | 1.6 |
| 0.211 | 1.8×10^{-5} | 1.8 |
| 0.201 | 2×10^{-5} | 0.2 |

Table 7: The continuous variation method of erythromycin with metal (copper) complex.

| Absorbance at $\lambda = 404$ for Color complex | VD / VT | V M mL | V D mL |
|---|---------|--------|--------|
| 0.0245 | 0.1 | 0.9 | 0.1 |
| 0.078 | 0.2 | 0.8 | 0.2 |
| 0.14 | 0.3 | 0.7 | 0.3 |
| 0.187 | 0.4 | 0.6 | 0.4 |
| 0.262 | 0.5 | 0.5 | 0.5 |
| 0.209 | 0.6 | 0.4 | 0.6 |
| 0.175 | 0.7 | 0.3 | 0.7 |
| 0.111 | 0.8 | 0.2 | 0.8 |
| 0.056 | 0.9 | 0.1 | 0.9 |

VD: values of the compound (erythromycin)

V M: The values of the metal (copper).

VT: Total (V M+V D)

Conclusion

Exploring a method for determination of copper ion by erythromycin is an easy, safe and Preconcentration of trace metals in aqueous solutions. The ligand was successfully to formed complex with the copper metal ion. Is a stable, sensitive and selective complexation successfully to determination Cu (II) in some Pharmaceuticals, the method gives a very low limit of detection and good R.S.D.

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