

Spectrophotometric Determination of Cu(II) Via Complexation with Cefixime



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Abstract

An optimized and validated spectrophotometric method has been developed for the determination of cefixime in pharmaceutical formulations. The method is based on the complexation reaction between cefixime and palladium ion in the presence of acidic buffer solution (pH 3) in ethanol-distilled water medium at room temperature. The complex absorbed maximally at 352 nm. Beer's law is obeyed in the working concentration range of 2.5–35 µg/mL with apparent molar absorptivity of 1.015×10^4 L/mol cm and Sandell's sensitivity of 0.001 µg/cm²/0.001 absorbance unit. The limits of detection and quantitation for the proposed method are 0.175 and 0.583 µg/mL, respectively. The effect of common excipients used as additives has been studied in the determination of cefixime. The proposed method has been successfully applied for the determination of cefixime in pharmaceutical formulations. The results obtained by the proposed method were statistically compared with the reference method using *t* and *F* values and found no significant difference between the two methods.

Keywords: Spectrophotometric, Cefixime, Spectral Studies.

Introduction

Soil plays an important role in biogeochemical balance of the biosphere. Degradation of soil leads to a reduction or complete loss of its ecological and productive values. It is caused primarily by chemical pollution, especially with excessive, unnatural amounts of trace elements such as cadmium, lead, zinc and copper, which may disturb the function of the complex system of processes occurring in the soil, and cause negative changes in biological activity and physical properties of the soil [1-5]. Chemical degradation of soil is particularly fast when trace elements, which are introduced into it, form easily soluble compounds. Then, their concentration in the water phase of the soil increases and, consequently, their bioaccumulation and migration become easier. Pesticides act as ligands for metals present in the soil, form complex compounds with them. Many of these are readily soluble in water, get into the ground water and then, through rivers, into the reservoirs of drinking water.

Copper belongs to elements whose natural content in the soil is most considerably exceeded. Soils which are considered unpolluted may contain (in three classes of granulation and reaction) 5, 25 or 40 mg copper per kg of dry substance. In Poland no standards of soil pollution have been established, so the so-called normal concentration of the determined substance in unpolluted soils is frequently used for reference in determination of soil pollution. For copper, normal concentration is 8.8 mg per kg of soil. In polluted soils the value is 50 mg per kg and in contaminated soils it is over 200 mg per kg of soil. According to the guidelines of the Institute of Agriculture, Fertilisation and Pedology in Pulawy, the natural content of copper in the soil is from 15 to 40 mg per kg of dry substance, depending on the type of soil [6]. Copper is introduced to the environment from natural sources (about 4.0-108 kg per year) and as a result of human activity (about 4.5-109 kg per year). It is accumulated in the soil (especially in its surface layers) with all its negative consequences [7]. Copper is indispensable for normal development of living organisms, but both its excess and deficiency are harmful. Copper deficiency in the diet may cause anaemia, insufficient growth, fertility problems, nervous system disorders and circulatory system diseases. Its excess may lead to changes in the liver and damage kidneys, brain tissue, coronary vessels and myocardium. Air in large cities, polluted with car exhaust and dust, directly affects soil and cultivated plants [8,9]. Due to these reasons, it is important to analyze Cu(II) in soil and water samples. Cefixime

(CFX) 1 [(6*R*,7*R*,*E*)-7-(2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid], is considered as an important and active member of third generation cephalosporin. The cefixime exists in off white crystals, melts over 250–220 °C and soluble in alcohol [10].

Objective of the Study

Interaction of Cu(II) with cefixime. The Cu(II)-cefixime complex has been studied spectrophotometrically to determine the Cu(II) content in water samples.

Review of Literature

An orally active cefixime has excellent activity against pathogens such as, Anaerobes, Enterobacteriaceae, gram negative species such as *Escherichia coli*, *klebsiella*, *Haemophilus influenzae*, *Branhamella Catarrhalis*, *Neisseria gonorrhoeae*, *Serratiamarcescens*, *Providencia*, *Haemophilus* and *Meningococcus* including β -lactamase producing strains [10,11,12]. Along with its broad spectrum antimicrobial activity and stability, cefixime is considered as most convenient in appropriate dosage for adults as well as pediatrics and widely prescribed among cephalosporin family in Pakistan. Various analytical methods have been reported for analysis of cefixime and other antibiotics after complexation and derivatization with a variety of chemical reagents [12,13,14,15,16,17]. Cephalexin belongs to the first generation cephalosporins. Many drugs possess modified toxicological and pharmacological properties when they are complexed. In this series, metal complexes of Cu(II) and Zn(II) with Schiff base of cephalexin (Salicylidene cephalexin) of the type, ML₂, have been prepared. The conductometric titration revealed that one mole of metal complexed with two moles of ligand. The physical, analytical and spectral studies of Schiff base and its complexes confirmed that the coordination of metal to the Schiff base occurred through phenolic deprotonated oxygen and the imino nitrogen [18]. Nowadays, the selective and sensitive monitoring of trace metal ions from different sources especially in water have become more important due to their toxic effects on environmental and biological systems. Copper and its salts are used in industries, laboratories, medicines, foods and beverages. Therefore, it is significant to develop uncomplicated, rapid and effective methods for quantitative analysis of copper in food, industrial and environmental samples. For copper(II) determination, several analytical techniques have been used such as atomic absorption spectrometry [19-21], voltammetry [22,23], potentiometry [24-28], inductive coupled plasma-emission spectrometry [29] and inductive coupled plasma-mass spectrometry [30]. Spectrophotometry is the widely used analytical technique in analysis because it is simple, economic, and easily available to most laboratories. Many of spectrophotometric reagents have been used for determination of Cu(II). However, most of these reagents withdraw from various limitations such as more time for color development, heating, narrow Beer's range, interferences from many ions [31-38]

Experimental

UV-visible spectrophotometric spectral studies of Cu(II), cefixime and Cu(II)-cefixime complex

Apparatus

All spectral and absorbance measurements were made on a Shimadzu UV-visible 1601 spectrophotometer (Kyoto, Japan).

Reagents and Standards

All reagents used were of analytical reagent grade.

1. 3.195×10^{-3} M copper sulphate (M.W.: 159.61, Suprachim products Ltd, England) solution (203.06 ppm Cu) was prepared by dissolving 0.051 g in 100 mL distilled water.
2. 2.205×10^{-3} M (0.1%) cefixime (CAS: 79350-37-1, M.W.: 453.45, Merck, USA) solution was freshly prepared in methanol.

Procedure

UV Spectrum of Copper Sulphate

2.0 mL of 3.195×10^{-3} M copper sulphate solution was taken in 10 mL standard volumetric flask and diluted up to the mark with distilled water. The UV spectrum of the copper sulphate solution was recorded in the wavelength range of 200-400 nm against distilled water as the blank solution. The solution is used to get the maximum absorption wavelength of the copper sulphate solution.

UV Spectrum of Cefixime

0.05 mL of 2.205×10^{-3} M cefixime was taken in 10 mL standard volumetric flask and diluted up to the mark with methanol. The UV spectrum of the drug solution was recorded in the wavelength range of 200-400 nm against methanol as the blank solution to get the λ_{max} of the drug solution.

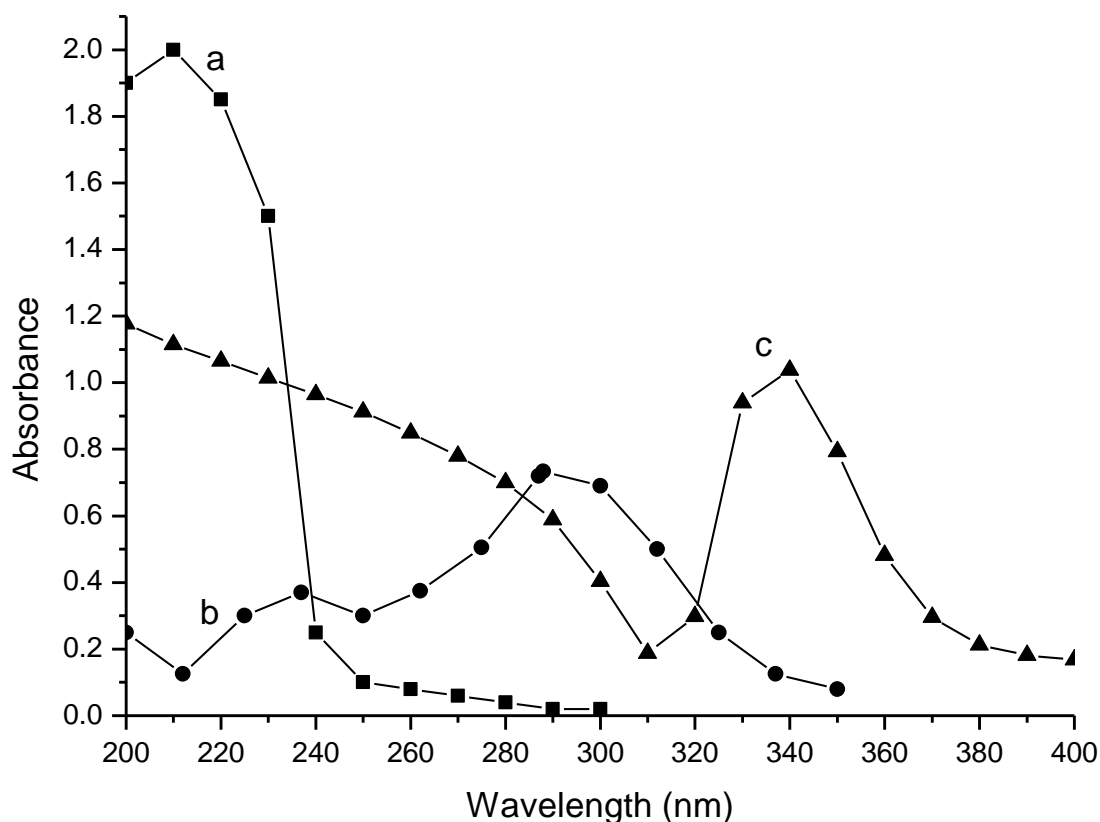
UV Spectrum of cefixime-Cu(II) Complex

0.4 mL of 3.195×10^{-3} M copper sulphate solution was added with 1.7 mL of 2.205×10^{-3} M cefixime into a 10 mL standard volumetric flask. The contents of the flask were mixed well at room temperature and diluted up to the mark with ethanol. The absorption spectrum was recorded in the wavelength range of 200-400 nm against the reagent blank prepared similarly except Cu(II) to get the λ_{max} of the cefixime-Cu(II) complex. The stability of the colored complex should also be checked.

Results and Discussion

The aqueous solution of copper sulphate was maximally absorbed at 206 nm. The methanol solution of cefixime was peaking at 290 and 210 nm. When the two solution were added, a red shift in the wavelength is observed due to the complexation reaction of Cu(II) with cefixime. Thus, a complex was obtained with λ_{max} of 336 nm. The UV spectra for copper sulphate, cefixime and Cu(II)-cefixime complex were shown in Fig. 2.1. The reaction was carried out at room temperature and the coloured complex was stable up to 6 h.

Fig.1. Absorption Spectra Of (A) 2 MI Of 3.195×10^{-3} M Copper Sulphate In Distilled Water (B) 0.05 MI Of 0.1 % Cefixime In Methanol And (C) 0.4 MI Of 3.195×10^{-3} M Copper Sulphate In Distilled Water + 1.7 MI Of 0.1% Cefixime In Methanol. Each Solution Is Diluted Up To The Mark With 1,4-Dioxan In 10 MI Standard Volumetric Flask.



Effect of the Concentration of Cefixime on the Absorbance of Complex Formation between Cefixime And Copper Sulphate

Apparatus

Absorbance measurements were made on a Shimadzu UV-visible 1601 spectrophotometer (Kyoto, Japan).

Reagents and Standards

1. 3.195×10^{-3} M copper sulphate solution was prepared in distilled water.
2. 2.205×10^{-3} M (0.1%) cefixime (CAS: 79350-37-1, M.W.: 453.45, Merck, USA) solution was prepared in methanol.

Procedure

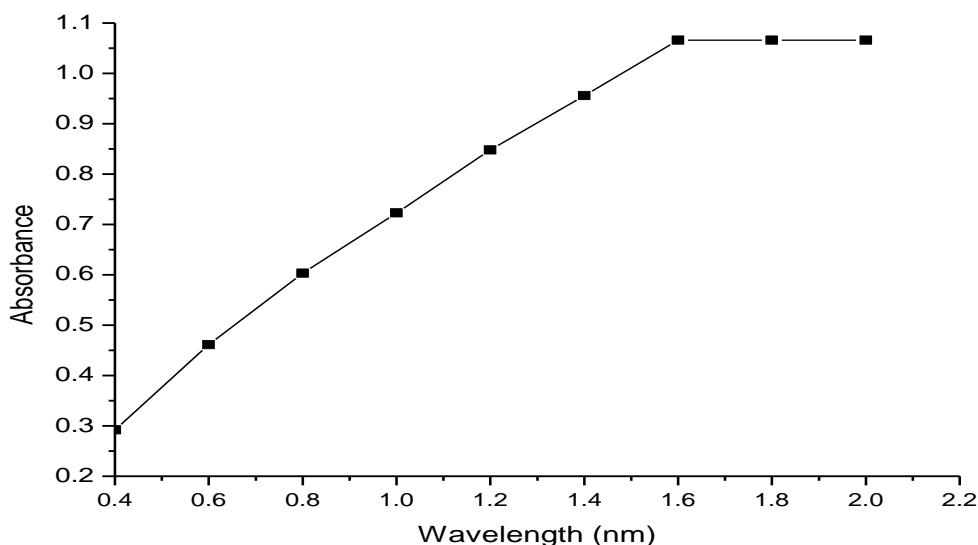
The effect of the volume of cefixime on the absorbance of the complex was investigated. For this purpose, 0.4 mL of 3.195×10^{-3} M copper sulphate solution corresponding to $8.122 \mu\text{g mL}^{-1}$ was added

with varied volumes (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mL) of 0.1% cefixime (2.205×10^{-3} M) and diluted with 1,4-dioxan in 10 mL standard volumetric flask. The absorbance was recorded at 336 nm against the reagent blank prepared similarly except Cu(II).

Results and Discussion

The volume of 0.1% cefixime was optimized for the development of the complex by recording the absorbance at 336 nm. The effect of the volume of cefixime on the absorbance of complex was examined in the range 0.4-2.0 mL 0.1% cefixime. It is clear from Fig. 2.2 that the maximum absorbance was obtained with 1.6 mL cefixime. Above this volume up to 2.0 mL of 0.1% cefixime, the absorbance remained unchanged. Therefore, 1.8 mL of 0.1% cefixime was used in the determination of Cu(II).

Fig. 2. Effect of the Volume of 0.1% Cefixime



Effect of Solvents on The Absorbance of The Cu(II)-Cefixime Complex

Apparatus

All absorbance measurements were made on a Shimadzu UV-visible 1601 spectrophotometer (Kyoto, Japan).

Reagents and Standards

1. 3.195×10^{-3} M copper sulphate solution was prepared in distilled water.
2. 2.205×10^{-3} M (0.1%) cefixime (CAS: 79350-37-1, M.W.: 453.45, Merck, USA) solution was prepared in methanol.
3. Solvents such as methanol, acetone, dimethylsulphoxide, acetonitrile, ethanol, 1,4-dioxan and distilled water were investigated for the best solvent in the determination process of the complex.

Procedure

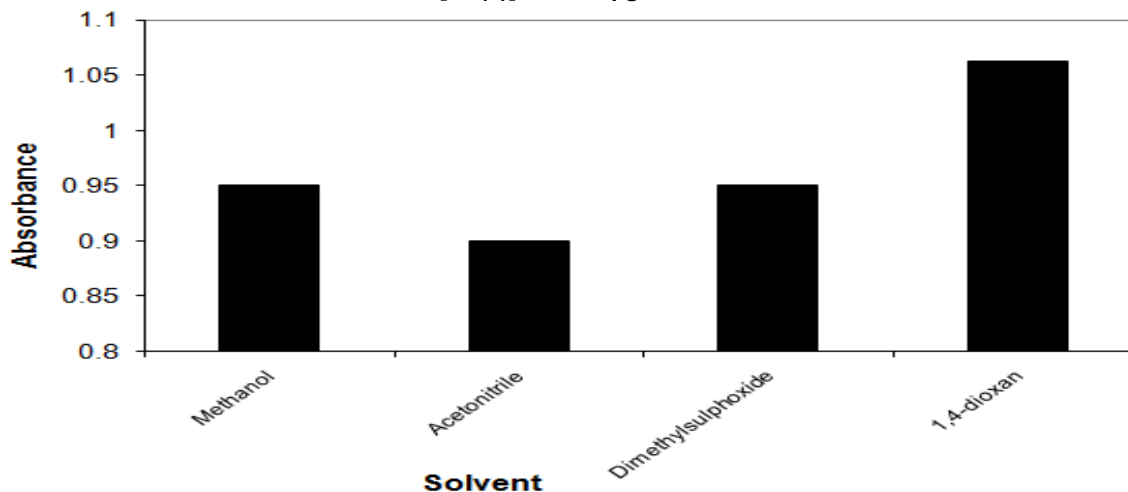
Aliquot of 0.4 mL of 3.195×10^{-3} M copper sulphate solution was added with 1.8 mL of 0.1%

cefixime and diluted with different solvents (methanol, acetone, dimethylsulphoxide, acetonitrile, ethanol, 1,4-dioxan and distilled water) in 10 mL standard volumetric flask. The absorbance was recorded at 504 nm for the maximum absorbance in the respective solvent.

Results and Discussion

The effect of solvents such as methanol, acetone, dimethylsulphoxide, acetonitrile, ethanol, 1,4-dioxan and distilled water were investigated on the absorbance of the Cu(II)-cefixime complex. The reaction mixture was turbid in ethanol, acetone and distilled water. The absorbance for Cu(II)-piroxicam complex in other solvents is shown in Fig. 2.3. It is evident from the figure that the highest absorbance was obtained in 1,4-dioxan. Therefore, 1,4-dioxan is selected as the best solvent for the dilution of the reaction mixture of Cu(II)-piroxicam.

Fig. 3. Effect of the solvent on the absorbance of the Cu(II)-cefixime complex, [Cu(II)] = $8.122 \mu\text{g mL}^{-1}$



pH of the Cu(II)-Cefixime Complex**Apparatus**

Hanna pH meter with pH and potential mode.

Reagents and standards

1. 3.195×10^{-3} M copper sulphate solution was prepared in distilled water.
2. 2.205×10^{-3} M (0.1%) cefixime (CAS: 79350-37-1, M.W.: 453.45, Merck, USA) solution was prepared in methanol.

Procedure

Aliquot of 1.2 mL of 3.195×10^{-3} M copper sulphate solution was added with 5.4 mL of 2.205×10^{-3} M (0.1%) cefixime and diluted with 1,4-dioxan in 10 mL standard volumetric flask and the pH of the reaction mixture is recorded.

Results and discussion

The pH of the reaction product was investigated and found to be 4.68. The pH of the reaction mixture is acidic.

Linearity of the Cu(II)-cefixime complex and the calibration graph**Apparatus**

Absorbance measurements were made on a Shimadzu UV-visible 1601 spectrophotometer (Kyoto, Japan).

Reagents and standards

1. 3.195×10^{-3} M copper sulphate solution was prepared in distilled water.

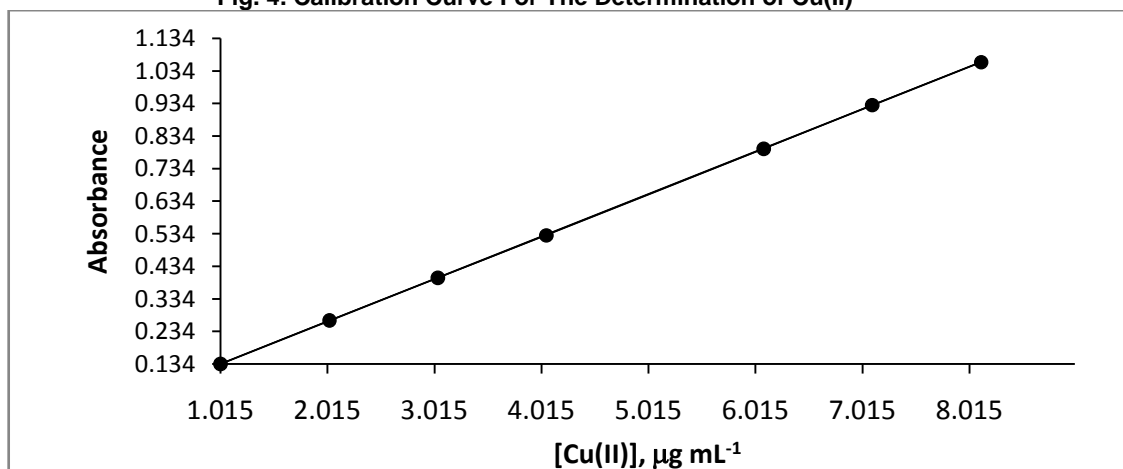
2. 2.205×10^{-3} M (0.1%) cefixime (CAS: 79350-37-1, M.W.: 453.45, Merck, USA) solution was prepared in methanol.

Procedure

Into a series of standard volumetric flask, different volumes (0.05, 0.1, 0.15, 0.2, 0.3, 0.35 and 0.4 mL of 3.195×10^{-3} M copper sulphate solution corresponding to 1.015, 2.031, 3.046, 4.061, 6.092, 7.107 and 8.122 $\mu\text{g mL}^{-1}$ were added with 1.8 mL of 2.205×10^{-3} M cefixime solution. The contents of the reaction mixture were mixed well and diluted up to the mark with 1,4-dioxan. The absorbance was measured at 366 nm against the reagent blank prepared similarly except Cu(II). The amount of Cu(II) in real samples can be calculated either from a calibration graph or regression equation.

Results and Discussion

Calibration curve was constructed by plotting the absorbance against the concentration of Cu(II) and found to be linear over the concentration range 1.015 - 8.122 $\mu\text{g mL}^{-1}$ (Fig. 2.5) with molar absorptivity of $8.293 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$. Optical, analytical and statistical parameters of the experimental data such as regression equation computed from calibration graph, correlation coefficient (r), detection limit and quantitation limit are summarized in Table 2.1. The high value of correlation coefficient (0.9999) for the proposed method indicated excellent linearity.

Fig. 4. Calibration Curve For The Determination of Cu(II)**Table 1. Optical and Analytical Parameters of The Proposed Method**

Parameters	Analytical data
λ_{max} (nm)	336 nm
Beer's law limit ($\mu\text{g mL}^{-1}$)	1.015 - 8.122
Molar absorptivity ($\text{L mol}^{-1} \text{ cm}^{-1}$)	8.293×10^3
Sandell's sensitivity	$0.008 \mu\text{g/cm}^2 / 0.001$ absorbance unit
Linear regression equation ^a	$A = 1.59 \times 10^{-3} + 1.305 \times 10^{-1} C$
S_a	9.850×10^{-4}
$\pm tS_a$	2.533×10^{-3}
S_b	1.919×10^{-5}
$\pm tS_b$	4.933×10^{-3}
Correlation coefficient (r)	0.9999
Variance (S_o^2)	1.588×10^{-6}
LOD ($\mu\text{g mL}^{-1}$)	0.032
LOQ ($\mu\text{g mL}^{-1}$)	0.097

^aWith respect to $A = a + bC$, where C is the concentration in $\mu\text{g mL}^{-1}$ and A is absorbance.

$\pm S_a$ and $\pm S_b$ are the confidence limits for intercept and slope, respectively.

Molar Combining Ratio of Cu(II) and Cefixime for The Formation Of The Complex

Apparatus

Absorbance measurements were made on a Shimadzu UV-visible 1601 spectrophotometer.

Reagents and Standards

All reagents used were of analytical reagent grade.

- 0.035 % Cu solution (2.205×10^{-3} M) was prepared in distilled water.
- 2.205×10^{-3} M (0.1%) cefixime was freshly prepared in methanol.

Procedure for the Molar Combining Ratio of The Complex

The stoichiometry of the reaction was studied by Job's method of continuous variations [23]. For this purpose, different volumes (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.35, 1.5, 1.8, 2.0 mL) of 2.205×10^{-3} M copper sulphate was added with different volumes (2.0, 1.8, 1.6, 1.4, 1.0, 0.8, 0.65, 0.5, 0.2, 0 mL) of 2.205×10^{-3} M cefixime and diluted with 1,4-dioxan in 10 mL standard volumetric flask. The absorbance was recorded at 504 nm and was plotted against the mole fraction of copper sulphate.

Results and Discussion

Stoichiometry of reaction between Cu(II) and cefixime was evaluated by Job's method of continuous variations wherein Cu(II) and cefixime solutions with identical molar concentrations are

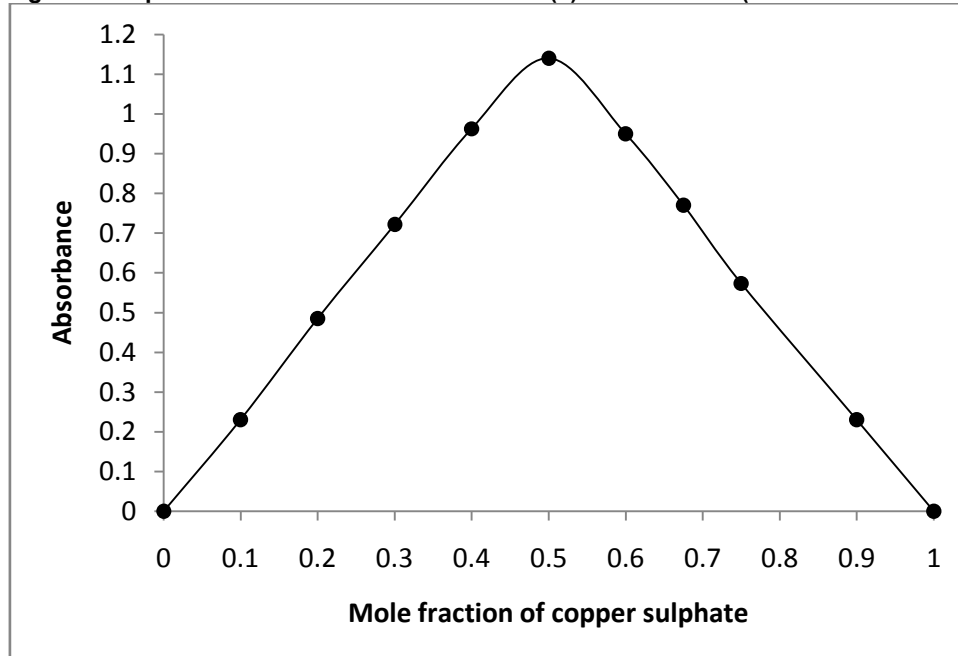
varied but the total volume remained constant. Absorbance of each determination is recorded and plotted against the mole fraction of Cu(II) (number of moles of copper sulphate / number of moles of copper sulphate + number of moles of cefixime) on x-axis is plotted (Fig. 2.6). The maximum absorbance is obtained at 1.0 mL of 2.205×10^{-3} M Cu(II) and 1.0 mL of 2.205×10^{-3} M cefixime. It is clear from the figure that 1 mol of Cu(II) reacted with 1 mol of cefixime. The resulting metal chelate remained stable for about 6 h. Thus, the combining molar ratio between Cu(II) and cefixime is 1:1.

The formation constant (K_f) for the complex formation between Cu(II) and cefixime is calculated using the following expression:

$$K_f = \frac{(A_{obs}/A_{extp})\bar{C}}{[C_M - (A_{obs}/A_{extp})\bar{C}][C_L - (A_{obs}/A_{extp})\bar{C}]}$$

Where A_{obs} and A_{extp} are observed and extrapolated absorbance values for the complex, respectively. C_M and C_L are the initial concentration of Cu(II) and cefixime in mol L^{-1} , respectively. \bar{C} is the limiting concentration. Thus, K_f for the complex is found to be 1.723×10^6 . The apparent Gibbs free energy (ΔG°) was calculated using $\Delta G^\circ = -2.303 RT \log K_f$ and found to be $-35.59 \text{ kJ mol}^{-1}$ confirming the feasibility of the reaction.

Fig. 5 Job's plot of Continuous Variations of Cu(II) and Cefixime (2.205×10^{-3} M each)



Effect of metal ions on the absorbance of the Cu(II)-cefixime complex

Apparatus

Absorbance measurements were made on a Shimadzu UV-visible 1601 spectrophotometer (Kyoto, Japan).

Reagents and standards

- 3.195×10^{-3} M copper sulphate solution was prepared in distilled water.
- 2.205×10^{-3} M (0.1%) cefixime (CAS: 79350-37-1, M.W.: 453.45, Merck, USA) solution was prepared in methanol.

3. Metal ions such as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, CaCl_2 , $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and CuCl_2 (0.051 %) were dissolved in distilled water.

Procedure for the Determination of Cu(II) in the Presence of Metal Ions

Aliquot of 0.4 mL of 3.195×10^{-3} M copper sulphate solution was taken in 10 mL standard volumetric flask. To each flask, 1.8 mL of 0.1% cefixime was added with different volumes of 0.051% metal ions ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, FeCl_3 , $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, CaCl_2 , and $\text{MnSO}_4 \cdot \text{H}_2\text{O}$). The absorbance was recorded at 504 nm for no change in absorbance with different metal ions in the presence of Cu(II).

Specificity and selectivity of proposed method was evaluated by determining concentration of Fe(III) ($22.34 \mu\text{g ml}^{-1}$) in the presence of various metal ions added in the form of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, CaCl_2 , $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and CuCl_2 .

Results and Discussion

Varying concentrations of each ion with fixed concentration of Cu(II) were taken and absorbance was recorded to know concentration of Cu(II) ($8.122 \mu\text{g ml}^{-1}$). Tolerated amounts of each ion, whose concentration value is tested that caused less than $\pm 2\%$ absorbance alteration, has been found as follows: Fe^{2+} , 0.50; Cd^{2+} , 84.31; Pb^{2+} , 62.16; Zn^{2+} , 52.31; Mg^{2+} , 21.80 and Mn^{2+} , $27.47 \mu\text{g ml}^{-1}$. However, metal ions (Ni^{2+} , Al^{3+} , Ca^{2+} , and Cu^{2+}) interfere in determination of Cu(II).

Applicability of the presented method for the determination of Cu(II) in water samples

Apparatus

Absorbance measurements were made on a Shimadzu UV-visible 1601 spectrophotometer.

Reagents and standards

1. 3.195×10^{-3} M copper sulphate solution was prepared in distilled water.
2. 2.205×10^{-3} M (0.1%) cefixime (CAS: 79350-37-1, M.W.: 453.45, Merck, USA) solution was prepared in methanol.
3. Water samples collected from lake, tap, well, sea and treated industrial effluent water samples were collected different locations.

Procedure for the Determination of Cu(II) In Real Water Samples

Aliquot of 0.5 mL of different water samples was pipetted into a 10 mL standard volumetric flask with 1.8 mL of cefixime (0.1%) solution. The contents of each flask were mixed well at room temperature and diluted up to the mark with 1,4-dioxan. The absorbance of each real water sample solution was measured at 504 nm against the reagent blank prepared similarly except Cu(II). The amount of the Cu(II) in real water samples was obtained either from the calibration graph or the regression equation.

Results and Discussion

The applicability of the presented method for the determination of Cu(II) in real samples has been tested. The concentration of Cu(II) was obtained either from the calibration graph or the regression equation and found to be

..... in lake water sample
 in tap water sample
 in well water sample and
 in tap water sample

Conclusions

The presented spectrophotometric method is found to be simple, selective and accurate for determination of Cu(II) in water samples. The method has advantage of using 1,4-dioxan-distilled water medium. The presented method has avoided the use of acid, buffer solution and heating of reaction mixture, can be used as an alternate method for routine quality control analysis of Cu(II) in soil samples.

Endnotes

1. A. Kabata-Pendias, Wybrane zagadnienia związane z chemicznym zanieczyszczeniem gleb, Wyd. PAN 1989.
2. A. Kabata-Pendias, H. Pendias, Biogeochemia pierwiastkow sladowych, PWN, Warszawa 1993.
3. L. Pawlowski, Wiad. Chem. 43 (1984) 451.
4. F. Maciak, Ochrona i rekultywacja srodowiska, Wyd. SGGW, Warszawa, 1996.
5. B. Jankiewicz, B. Ptaszynski, A. Turek, Polish J. Env. Studies 8 (1999) 35.
6. A. Wachwik, P. Z. H, Rocz 38 (1987) 363.
7. T. Golda, S. Gruszczynski, M. Trafas, Arch. Ochr. Srod. 3-4 (1994) 171.
8. H. Terelak, i inn. Arch. Ochr. Srod. 3-4 (1997) 167.
9. I. Szczechinski, H. Gozinski, I. Sikora, M. Kleczkowski, Bromat. Chem. Toksykol. 16 (1986) 3.
10. Troy, D.B., Beringer, P., 2005. Remingtons, the science and practice of pharmacy. 21st ed., vol. 2, pp. 1644, May 19, ISBN-10: 0781746736.
11. Katzung, B., 2006. Basic and clinical pharmacology, (Chapter 8), Chemotherapeutic Drugs tenth ed., p. 726, ISBN-10: 0071451536.
12. Sayed N.H.A., Bashir I., Nada S.H.A., Iman R.S.A., Noora A.S.A., Nafisur R. Quantitative analysis of cefixime via complexation with palladium(II) in pharmaceutical formulation by spectrophotometry. J. Pharm. Anal. 2013; 3(4): 248–256.
13. El-Shaboury S.R., Saleh A.G., Mohamed F.A., Rageh A.H. Analysis of cephalosporin antibiotics. J. Pharm. Biomed. Anal. 2007;45(1):1–19.
14. Wani Y.B., Patil D.D. An experimental design approach for optimization of spectrophotometric method for estimation of cefixime trihydrate using ninhydrin as derivatizing reagent in bulk and pharmaceutical formulation. J. Saudi. Chem. Soc. 2013
15. Ahmed S.M.A., El-bashir A.A., Aboul-Enein H.Y. New spectrophotometric method for

- determination of cephalosporins in pharmaceutical formulations. *Arab.J. Chem.* 2011
16. Adegoke O.A., Quadri M.O. Novel spectrophotometric determinations of some cephalosporins following azo dye formation with *p*-dimethylaminobenzaldehyde. *Arab.J. Chem.* 2012
 17. Adegoke O.A., Umoh O.E. A new approach to the spectrophotometric determination of metronidazole and tinidazole using *p*-dimethylaminobenzaldehyde. *Acta Pharm.* 2009;59(4):407-419.
 18. Saurabh Sharma, Neeraj kumar, Shanili gupta. Metal ion interaction with electron rich species. *International journal of current research* Vol. 10, Issue, 03, pp.66271-66285, March, 2018
 19. R.J. Cassella, O.I.B. Magalhaes, M.T. Couto, E.L.S. Lima, M.A.F.S. Neves, F.M.B. Coutinho, Synthesis and application of a functionalized resin for flow injection/F AAS copper determination in waters, *Talanta* 67 (2005) 121-128.
 20. M.H. Mashhadizadeh, M. Pesteh, M. Talakesh, I. Sheikhshoae, M. Mazloum-Ardakani, M.A. Karimi, Solid phase extraction of copper (II) by sorption on octadecyl silica membrane disk modified with a new Schiff base and determination with atomic absorption spectrometry, *Spectrochim Acta B* 63 (2008) 885-888.
 21. C.A. Sahin, I. Tokgoz, A novel solidified floating organic drop microextraction method for preconcentration and determination of copper ions by flow injection flame atomic absorption spectrometry, *Anal Chim Acta* 667 (2010) 83-87.
 22. A. Mohadesi, M.A. Taher, Voltammetric determination of Cu(II) in natural waters and human hair at a meso-2,3-dimercaptosuccinic acid self-assembled gold electrode, *Talanta* 72 (2007) 95-100.
 23. H. Bagheri, A. Shirzadmehr, M. Rezaei, Determination of copper ions in foodstuff products with a newly modified potentiometric carbon paste electrode based on a novel nano-sensing layer, *Ionics* (2016) 1-12.
 24. M. Andac, F. Coldur, S. Bilir, A. Birinci, S. Demir, H. Uzun, Solid-contact polyvinyl chloride membrane electrode based on the bis[2-(hydroxyethylimino)phenolato]copper(II) complex for trace level determination of copper ions in wastewater, *Can J Chem* 92 (2014) 324-328.
 25. M. Ghanei-Motlagh, M.A. Taher, V. Saheb, M. Fayazi, I. Sheikhshoae, Theoretical and practical investigations of copper ion selective electrode with polymeric membrane based on N,N'-(2,2-dimethylpropane-1,3-diyl)-bis(dihydroxyacetophenone), *Electrochim Acta* 56 (2011) 5376-5385.
 26. A. Islamnezhad, N. Mahmoodi, Potentiometric Cu(II)-selective electrode with subnanomolar detection limit, *Desalination* 271 (2011) 157-162.
 27. Y.M. Issa, H. Ibrahim, O.R. Shehab, New copper(II)-selective chemically modified carbon paste electrode based on etioporphyrin I dihydrobromide, *J Electroanal Chem* 666 (2012) 11-18.
 28. A. Shokrollahi, A. Abbaspour, M. Ghaedi, A.N. Haghghi, A.H. Kianfar, M. Ranjbar, Construction of a new Cu(II) coated wire ion selective electrode based on 2-((2-(2-(2-(2-hydroxy-5-methoxybenzylideneamino)phenyl)disufanyl)phenyl)mino)methyl)-4-methoxyphenol Schiff base, *Talanta* 84 (2011) 34-41.
 29. T. Kamiura, K. Funasaka, Y. Tajima, T. Kawaraya, K. Kuroda, Pretreatment by yeast for determination of nickel and vanadium in bitumen-in-water emulsion by inductively coupled plasma atomic emission spectrometry, *Anal Chim Acta* 327 (1996) 61-64.
 30. A. Martin-Camean, A. Jos, M. Puerto, A. Calleja, A. Iglesias-Linares, E. Solano, A.M. Camean, In vivo determination of aluminum, cobalt, chromium, copper, nickel, titanium and vanadium in oral mucosa cells from orthodontic patients with mini-implants by Inductively coupled plasma-mass spectrometry (ICP-MS), *J Trace Elem Med Bio* 32 (2015) 13-20.
 31. F.J. Huo, C.X. Yin, Y.T. Yang, J. Su, J.B. Chao, D.S. Liu, Ultraviolet-Visible Light (UV-Vis)-Reversible but Fluorescence-Irreversible Chemosensor for Copper in Water and Its Application in Living Cells, *Anal Chem* 84 (2012) 2219-2223.
 32. F. Dayou, Y. Dong, Spectrophotometric determination of trace copper in water samples with thiomichlersketone, *Spectrochim. Acta A* 66 (2007) 434-437
 33. J.Y. Kwon, Y.J. Jang, Y.J. Lee, K.M. Kim, M.S. Seo, W. Nam, J. Yoon, A highly selective fluorescent chemosensor for Pb²⁺, *J Am Chem Soc* 127 (2005) 10107-10111.
 34. Q. Lin, P. Chen, J. Liu, Y.P. Fu, Y.M. Zhang, T.B. Wei, Colorimetric chemosensor and test kit for detection copper(II) cations in aqueous solution with specific selectivity and high sensitivity, *Dyes Pigments* 98 (2013) 100-105.
 35. J.H. Soh, K.M.K. Swamy, S.K. Kim, S. Kim, S.H. Lee, J. Yoon, Rhodamine urea derivatives as fluorescent chemosensors for Hg²⁺, *Tetrahedron Lett* 48 (2007) 5966-5969. H. Yang, Z.G. Zhou, K.W. Huang, M.X. Yu, F.Y. Li, T. Yi, C.H. Huang, Multisignaling optical-electrochemical sensor for Hg²⁺ based on a rhodamine derivative with a ferrocene unit, *Org Lett* 9 (2007) 4729-4732.
 36. Z.Y. Yao, B.H. Huang, X.P. Hu, L. Zhang, D.P. Li, M. Guo, X.H. Zhang, H. Yuan, H.C. Wu, Colorimetric detection of copper ions based on a supramolecular complex of water-soluble polythiophene and ATP, *Analyst* 138 (2013) 1649-1652.
 37. L. Zeng, E.W. Miller, A. Pralle, E.Y. Isacoff, C.J. Chang, A selective turn-on fluorescent sensor for imaging copper in living cells, *J Am Chem Soc* 128 (2006) 10-11.
 38. Z. Cimerman, N. Galesic, B. Bosner, Structure and Spectroscopic Characteristics of Schiff-Bases of Salicylaldehyde with 2,3-Diaminopyridine, *J Mol Struct* 274 (1992) 131-144.
 39. O. Atakol, H. Nazir, M.N. Tahir, D. Ulku, Crystal structure of (N,N'-disalicylidene-2,3-diaminopyridine)copper(II) complex, *Anal Sci* 13 (1997) 519-520.