Communications

**UV Spectrophotometric Determination of Cu(II) in Synthetic Mixture and Water Samples**

Lutfullah, a* Saurabh Sharma, a Nafisur Rahman, a Syed Najmul Hejaz Azmi, b Bashir Iqbal, b Maisa Ismail Bilal Bait Amburk b and Zuweina Masoud Hilal Al Barwani b

a Department of Chemistry, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India
b Department of Applied Sciences, Chemistry Section, Higher College of Technology, P. O. Box 74, Al-Khuwair-133, Muscat, Sultanate of Oman

A new and novel UV spectrophotometric method has been developed for the determination Cu(II) in synthetic mixture and water samples. The method is based on complex formation of Cu(II) with cefixime immediately in 1,4-dioxan-distilled water medium at room temperature. The complex showed maximum absorption wavelength at 336 nm. Beer’s law is obeyed in the concentration range of 1.015-8.122 /c109 (Linear regression: A = 1.59 × 10^{-3} + 1.305 × 10^{-1} C) with apparent molar absorptivity (8.29 × 10^{3} L mol^{-1} cm^{-1}) and Sandell’s sensitivity (0.008 μg/cm²/0.001 absorbance unit). The limits of detection and quantitation for the developed method are 3.19 × 10^{-2} and 9.65 × 10^{-2} /c109, respectively. Interferences due to Mn(II), Ca(II), Mg(II), Cd(II), Al(III), Pb(II), Zn(II), Fe(II), Fe(III), Ni(II), Cr(III), Hg(II) and As(III) was investigated. Nine cations do not interfere in the determination of Cu(II). Proposed method was successfully applied to the determination of Cu(II) in synthetic mixture, sea and well water samples.

**Keywords:** UV Spectrophotometry; Validation; Cu(II); Cefixime; Cu(II)-cefixime complex; Sea and well water samples.

**INTRODUCTION**

Copper has received considerable attention owing to its technological and biological significance. It is an essential constituent of about thirty enzymes and glycoproteins. It is required for the synthesis of hemoglobin and for some biological processes. 1,2 The concentration of copper plays a very important role which decides its vitality and toxicity for many biological systems. 3,4 It has been reported that the toxic copper species are Cu(OH)_2, Cu_2(OH)_3 and CuCO_3. Therefore, Copper ions present in various aqueous systems are considered to be the most toxic of dissolved copper species. 1,2 The excess concentration of copper in water systems is harmful to human beings, affects the self-purification of bulk water 5 and disturbs the microbiological treatment of waste water. 6 Thus, the determination of trace amounts of Cu(II) is becoming increasingly important because of the increased interest in environmental pollution. 7 In chemical analysis, metal chelation followed by solvent extraction and spectrophotometric determination is considered as the most preferred mode of analysis for a number of metal ions in different matrices. 8 Several spectrophotometric methods have been reported in which the solvent extraction step is replaced by the use of surfactant. 9-13 Micellar media are mainly used to simplify the system and replacing the extraction step. Many spectrophotometric methods have been developed for the determination of Cu(II) based on reaction with reagents such as 1-nitroso-2-naphthol, 14 1-phenyl-1,2-propanedione-2-oxime thiosemicarbazone, 15 2-hydroxy-1-naphthaldehyde benzoylhydrazone, 16 N-ethyl-2-naphthalamine, 17 ethyl violet, 18 mixture of 1,10-phenanthroline and neocuproine, 19 1,5-diphenyl carbazole, 20 2-ketobutyric acid thiosemicarbazone, 21 pyruvic acid thiosemicarbazone, 22 2-acetyl thiophene-4-phenyl-3-thiosemicarbazone, 23 4-vanillidene amino-3-methyl-5-mercapto-1,2,4-triazole, 24 dimethyl glyoxime, 25 variamine blue. 26 Most of the reported spectrophotometric methods are time consuming, employing many reagents to develop the colour and extraction of copper complex into organic solvent. Spectrophotometry is the good tool for determining metal ion concentration in leafy vegetables, pharmaceuticals, natural water and soil samples due to its low cost, simplicity and adaptability. Analytical methods based on spectr-
photometry were published in reputed journals. Therefore, it is decided to exploit this technique to develop an optimized and validated UV spectrophotometric method for the determination of Cu(II) in synthetic mixture and water samples. The present UV spectrophotometric method is based on the complex formation of Cu(II) with cefixime in 1,4-dioxan-distilled water medium at room temperature (25 ± 1°C). The formed complex showed maximum absorbance at 336 nm. The reaction conditions are optimized and validated as per International Conference on Harmonisation (USA).

EXPERIMENTAL

Apparatus

All spectral and absorbance measurements were made on a Shimadzu UV-visible 160 A spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with 1 cm matched quartz cells.

IR spectra were recorded on a Perkin-Elmer FTIR 1650 spectrophotometer in wave number region 4000-400 cm⁻¹ using KBr pellet technique.

An Elico LI 120 pH meter (Hyderabad, India) was used for pH measurement.

Reagents and standards

All reagents used were of analytical reagent grade.

- 3.195 × 10⁻³ M copper sulphate (M.W.: 159.61, Suprachem products Ltd, England) solution (203.06 ppm Cu) was prepared by dissolving 0.051 g in 100 mL distilled water.

- 2.205 × 10⁻³ M (0.1%) cefixime (CAS: 79350-37-1, M.W.: 453.45, Sigma Chemical Company, St. Louis, USA) solution was freshly prepared in methanol. The solution was stable up to 12 h.

- Buffer solutions ranging from 3.72-5.57 were prepared by mixing varying volumes of 0.2 M acetic acid (9-1 mL) and 0.2 M sodium acetate (1-9 mL) in 10 mL standard volumetric flask.

- Sea and well water samples were collected from Muscat, Oman.

Procedure for the Determination of Cu(II)

Into a series of 10 mL standard volumetric flask, aliquots (0.05-0.4 mL) of 3.195 × 10⁻³ M standard copper sulphate aqueous solution corresponding to 1.015-8.122 μg mL⁻¹ were pipetted. To each flask, 1.8 mL of cefixime (0.1%) solution was added and diluted up to the mark with 1,4-dioxan. The contents of the flask were mixed well and the absorbance was measured at 336 nm against reagent blank prepared similarly except Cu(II) within stability period (6 h). The amount of Cu(II) was obtained either from the calibration graph or the regression equation.

Determination of Cu(II) in Synthetic Mixture Sample

Synthetic mixture of Cu(II) sulphate sample solution was prepared by taking 51 mg of copper sulphate with 200 mg MgSO₄, 7.28 mg HgCl₂, 200 mg Cr₂O₃, 290 mg Al₂(SO₄)₃.16H₂O, 51 mg ZnSO₄.7H₂O, 51 mg MnSO₄. H₂O, 51 mg CaCl₂, 51 mg Pb(NO₃)₂ and 72.8 mg Fe₂(SO₄)₃ in 100 mL standard volumetric flask and diluted up to the mark with distilled water. The amount of Cu(II) was determined by the proposed procedure.

Determination of Cu(II) in Water Samples

100 mL of sea and well water samples were collected and transferred into cleaned polyethylene bottles. The sea and the well water samples were filtered through a Millipore 0.45 μm pore size membrane and analyzed within 6 h of collection. Aliquot (0.5 mL) of sea and well 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 solution was recorded at 336 nm against the reagent blank prepared similarly except Cu(II). The amount of Cu(II) in sea and well water samples was obtained either from the calibration graph or the regression equation.

Determination of stoichiometry

The stoichiometry of the reaction was studied by Job’s method of continuous variations. 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 $2.205 \times 10^{-3}$ 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 $2.205 \times 10^{-3}$ M cefixime and diluted with 1,4-dioxan in 10 mL standard volumetric flask. The absorbance was recorded at 336 nm and plotted against the mole fraction of copper sulphate.

**Validation**

The present method has been validated for specificity, precision, accuracy, linearity, robustness and evaluation of bias.

The specificity of the proposed method was evaluated by determining 7.107 µg mL$^{-1}$ Cu(II) in the presence of metal ions such as Mn(II), Cu(II), Mg(II), Cd(II), Al(III), Pb(II), Zn(II), Fe(II), Fe(III), Ni(II), Cr(III), Hg(II) and As(III) added in the form of MnSO$_4$, H$_2$O, CaCl$_2$, MgSO$_4$, 3CdSO$_4$, 8H$_2$O, Al$_2$(SO$_4$)$_3$, 16H$_2$O, Pb(NO$_3$)$_2$, ZnSO$_4$, 7H$_2$O, FeSO$_4$, 7H$_2$O, Fe$_3$(SO$_4$)$_3$, Ni(NO$_3$)$_2$, 6H$_2$O, Cr$_2$O$_3$, HgCl$_2$, and As$_2$O$_3$.

The precision of the proposed method was evaluated by intra-day and inter-day precisions. Standard Cu(II) solution at three concentration levels (2.031, 4.061 and 8.122 µg mL$^{-1}$) was assessed with five replicates ($n = 5$) for each of three working sample concentrations in a single day (intra-day precision) and over five days (inter-day precision).

The accuracy of the proposed method was determined by standard addition method. For this purpose, 0.15 mL synthetic mixture sample solution corresponding to 3.046 µg mL$^{-1}$ Cu(II) was spiked with 0, 0.05, 0.1, 0.15 and 0.2 mL standard Cu(II) solution corresponding to 0, 1.015, 2.031, 3.046 and 4.061 µg mL$^{-1}$ Cu(II).

The robustness of proposed method was assessed by analyzing 5 µg mL$^{-1}$ Cu(II) in synthetic mixture sample by varying the volume of cefixime (1.8 ± 0.2 mL) at room temperature (25 ± 1°C).

Linearity of the proposed method was assessed at seven concentration levels (1.015, 2.031, 3.046, 4.061, 6.092, 7.107 and 8.122 µg mL$^{-1}$). Each concentration level was independently analyzed repeatedly for five times. The instrumental response i.e. absorbance obtained at each concentration was plotted against initial concentration of Cu(II). The linear regression equation was evaluated by least square treatment of the calibration data. The other statistical parameters for the proposed method were calculated using Origin Software. Limits of detection and quantitation were calculated.$^{34}$

Point and interval hypothesis tests have been utilized to evaluate the bias of the proposed method and the reference method.$^{35}$ The proposed method is compared with the reference method and considered to be acceptable if mean recovery of the proposed method is within ± 2.0% of that of the reference method. The lower ($t_{0.0}$) and the upper ($t_{0.0}$) acceptance limits can be calculated by the following quadratic equation:

$$
\theta^2 (x_1^2 - S_p^2 t_{0.0}^2 / n_1) + 0(-2x_1x_2) + (x_2^2 - S_p^2 t_{0.0}^2 / n_2) = 0
$$

where $\bar{x}_1$ and $\bar{x}_2$ are mean values at $n_1$ and $n_2$ measurements, respectively. $S_p$ is the pooled standard deviation and $t_{0.0}$ is the tabulated one-sided t-value at 95% confidence level.

**RESULTS AND DISCUSSION**

The aqueous solution of copper sulphate was maximally absorbed at 206 nm. The methanolic solution of cefixime was peaking at 210 and 290 nm. When the two solutions were mixed together, a red shift in the wavelength is observed due to the complexation reaction of Cu(II) and cefixime. Thus, a complex was obtained immediately in 1,4-dioxan-distilled water medium with $\lambda_{max} = 336$ nm. The UV spectra for copper sulphate, cefixime and Cu(II)-cefixime complex are shown in Fig. 1. It is clear from the graph that the maximum absorbance wavelength of the Cu(II)-cefixime complex is different to that of cefixime. Thus, the absorbance measurement at 336 nm as a function of initial concentration of Cu(II) is exploited to develop a new and novel UV spectrophotometric method for the determination of Cu(II) in synthetic mixture and water samples. The reaction was carried out at room temperature and the complex was stable up to 6 h.

**Stoichiometry**

The stoichiometric ratio between Cu(II) and cefixime was evaluated by Job’s method of continuous variations. Varied volumes of equimolar ($2.205 \times 10^{-3}$ M) solutions of Cu(II) and cefixime were taken, keeping the total volume of Cu(II) and cefixime constant at 2 mL in 10 mL standard volumetric flask and diluted up to the mark with 1,4-dioxan. The absorbance of each set is recorded and plotted against the mole fraction of Cu(II) (Fig. 2). This is due to the complexation reaction of Cu(II) with cefixime at 336 nm. The plot of absorbance versus mole fraction of Cu(II) has confirmed that 1 mol of Cu(II) reacted with 1 mol of
cefixime. The resulting Cu(II)-cefixime complex remained stable for about 6 h. Thus, the stoichiometry of the complex is established and found to be 1:1.

The I.R. spectra of free cefixime and Cu(II)-cefixime complex are shown in Fig. 3 a and b, respectively. Cefixime has -NH$_2$, -COOH, -CONH and C=O lactam groups which are the potential sites for coordination with metal ions. Comparison of IR spectrum of the complex with those of free cefixime indicates that the lactam (C=O) band appears at 1766 cm$^{-1}$ in the free cefixime while the complex shows this band again at 1766 cm$^{-1}$ suggesting that no coordination occurs with copper ion. The amide carbonyl band, (C=O)–NH in the free cefixime appears at 1674 cm$^{-1}$ with a weak shoulder at 1635 cm$^{-1}$ while the Cu(II)-cefixime complex shows this band at 1676 cm$^{-1}$ with a prominent peak at 1631 cm$^{-1}$ indicating the coordination of cefixime with Cu(II) through nitrogen. The asymmetrical and symmetrical stretching bands of carboxylate groups change from 1533 cm$^{-1}$ to 1543 cm$^{-1}$ and 1373 to 1379 cm$^{-1}$, respectively due to the coordination. The Cu-N stretching vibration occurs at 428 cm$^{-1}$. A tentative mechanism for the complexation between Cu(II) and cefixime is given in Scheme I.

**Scheme I**

Fig. 1. Absorption spectra of (a) 2 mL of 3.195 $\times$ 10$^{-3}$ M copper sulphate in distilled water (b) 0.05 mL of 0.1% cefixime in methanol and (c) 0.4 mL of 3.195 $\times$ 10$^{-3}$ M copper sulphate in distilled water + 1.8 mL of 0.1% cefixime in methanol. Solutions a, b and c are diluted up to mark with distilled water, methanol and 1,4-dioxan in 10 mL standard volumetric flask.

Fig. 2. Job’s plot of continuous variations for stoichiometric ratio between Cu(II) and cefixime (2.205 $\times$ 10$^{-3}$ M each).
The apparent formation constant ($K_f$) for the complex formation between Cu(II) and cefixime is calculated using the following expression:

$$K_f = \frac{(A_{\text{obs}} / A_{\text{exp}}) \bar{C}}{[C_M - \left(\frac{A_{\text{obs}}}{A_{\text{exp}}}\right)\bar{C}] [C_L - \left(\frac{A_{\text{obs}}}{A_{\text{exp}}}\right)\bar{C}]}$$

where $A_{\text{obs}}$ and $A_{\text{exp}}$ 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 $1.723 \times 10^6$. The apparent Gibbs free energy ($\Delta G^0_f$) is calculated using $\Delta G^0_f = -2.303 RT \log K_f$ and found to be $-35.59$ kJ mol$^{-1}$ confirming the feasibility of the reaction.

**Optimization of Variables**

The optimization of variables was investigated by testing reaction time, concentration of cefixime, solvents and sodium acetate-acetic acid buffer solutions of different pH.

The effect of reaction time on the absorbance of Cu(II)-cefixime complex and its stability was investigated. The Cu(II)-cefixime complex got stabilized immediately at 25 ± 1 °C after mixing cefixime and 1,4-dioxan as solvent. Complex remained stable for 6 h.

The volume of 0.1% cefixime was optimized for method development. The absorbance of the metal complex was investigated at 8.122 µg mL$^{-1}$ Cu(II) with volume of cefixime in the range 0.4-2.0 mL. It is clear from Fig. 4 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 further measurement of Cu(II) in synthetic mixture and water samples.

The effect of solvents such as methanol, acetone, dimethylsulphoxide (DMSO), acetonitrile, ethanol, 1,4-dioxan and distilled water was investigated at 8.122 μg mL⁻¹ of Cu(II) on the absorbance of Cu(II)-cefixime complex. The reaction mixture was turbid in ethanol, acetone and distilled water. The absorbance for Cu(II)-cefixime complex in other solvents is shown in Fig. 5. It is clear from the figure that the highest absorbance was obtained in 1,4-dioxan. Therefore, 1,4-dioxan was the best solvent for dilution of the reaction mixture of Cu(II)-cefixime complex in determination process of Cu(II) in synthetic mixture and water samples.

The pH of the complex was measured and found to be 4.68. The effect of pH on the absorbance of the complex was investigated using sodium acetate-HCl (range 3.72-5.57) buffer solutions. A constant absorbance was obtained in the pH range of 3.72-5.57, but the absorbance value in this pH range was found to be less than that obtained without buffer solution when dilution is made with 1,4-dioxan. Therefore, all absorbance measurements of the complex were made in 1,4-dioxan solvent without the involvement of buffer solution.

Validation

Varying concentrations of metal ions such as Mn(II), Ca(II), Mg(II), Cd(II), Al(III), Pb(II), Zn(II), Fe(II), Fe(III), Ni(II), Cr(III), Hg(II) and As(III) with 7.107 μg mL⁻¹ of Cu(II) were taken and the absorbance was recorded to know the concentration of Cu(II). The tolerated amount of each metal ion in μg mL⁻¹ was determined (Table 1), when the absorbance value did not exceed ± 2% on addition of cations. However, metal ions such as Ni(II), Cd(II), Fe(II) and As(III) interfere in determination of Cu(II).

The intra-day and inter-day precisions were evaluated by determining the concentration of Cu(II) at lower, middle and upper concentration levels for five repeated times within the same day and on five consecutive days, respectively (Table 2). It can be seen from the table that percentage recovery and RSD (intra day and inter day precisions) were in the ranges of 99.93-100.08% and 0.14-0.67%, respectively. It is evident from the table that percentage recovery and RSD values were precise and can be used to determine Cu(II) in synthetic mixture and water samples.

Standard addition method is one of the methods to show the accuracy of the proposed method just by the addition of the number of standardized aliquots to a real sample in order to raise its concentration by a known amount. Thus, the accuracy of the proposed method was investigated by performing recovery experiments through standard addition method. The absorbance for each solution is recorded at 336 nm and plotted as shown in Fig. 6. The re-
The results of analyses are summarized in Table 3. It is clear from the table and the graph that the linearity of the regression line for synthetic mixture sample was good. As can be seen from the figure that the concentration of Cu(II) in synthetic mixture sample is given by intercept/slope. The ratio of the intercept and the slope of the regression line is subjected to error (SxE), thus SxE is calculated from the following expression

\[ S_{xE} = \frac{S_y}{b} \left[ \frac{1}{n} + \frac{\sum (x_i - \bar{x})^2}{b^2} \right]^{1/2} \]

and found to be 0.02 \( \mu g \) mL\(^{-1}\). The confidence limit for the concentration of Cu(II) in synthetic mixture sample is calculated by \( x_E \pm tS_{xE} \) at \( n - 2 \) degrees of freedom and found to be 3.049 \( \pm 0.062 \). The most attractive feature of the proposed method using standard addition method is its relative freedom from various non targeted cations.

Table 1. Effect of metal ions on the determination of 7.107 \( \mu g \) mL\(^{-1}\) Cu(II)

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Added as</th>
<th>Tolerance limit (( \mu g ) mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg(^{2+})</td>
<td>MgSO(_4)</td>
<td>15.44</td>
</tr>
<tr>
<td>Hg(^{2+})</td>
<td>HgCl(_2)</td>
<td>1.88</td>
</tr>
<tr>
<td>Cr(^{3+})</td>
<td>Cr(_2)O(_3)</td>
<td>26.25</td>
</tr>
<tr>
<td>Al(^{3+})</td>
<td>Al(_2)(SO(_4))(_3)•16H(_2)O</td>
<td>4.36</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>ZnSO(_4)•7H(_2)O</td>
<td>3.48</td>
</tr>
<tr>
<td>Mn(^{2+})</td>
<td>MnSO(_4)•H(_2)O</td>
<td>4.97</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>CaCl(_2)</td>
<td>5.50</td>
</tr>
<tr>
<td>Pb(^{2+})</td>
<td>Pb(NO(_3))(_2)</td>
<td>12.76</td>
</tr>
<tr>
<td>Fe(^{3+})</td>
<td>Fe(_2)(SO(_4))(_3)</td>
<td>3.56</td>
</tr>
</tbody>
</table>

Table 2. Precision of the proposed method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intra day assay</th>
<th>Inter day assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration taken, ( \mu g ) mL(^{-1})</td>
<td>2.031</td>
<td>2.031</td>
</tr>
<tr>
<td>Concentration found, ( \mu g ) mL(^{-1})</td>
<td>2.023</td>
<td>2.033</td>
</tr>
<tr>
<td>Standard deviation(^2), ( \mu g ) mL(^{-1})</td>
<td>0.012</td>
<td>0.014</td>
</tr>
<tr>
<td>Recovery, %</td>
<td>99.93</td>
<td>99.95</td>
</tr>
<tr>
<td>Relative standard deviation, %</td>
<td>0.57</td>
<td>0.31</td>
</tr>
</tbody>
</table>

\(^a\) Mean for five independent analysis.
\(^b\) Confidence limit at 95\% confidence level and four degrees of freedom (\( t = 2.776 \)).

Table 3. Test of accuracy in synthetic mixture sample by standard addition method

<table>
<thead>
<tr>
<th>Concentration (( \mu g ) mL(^{-1}))</th>
<th>Linear regression parameters</th>
<th>Recovery(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Standard Added Nominal Error (( S_{xE} )) Intercept Slope ( r^2 )</td>
<td>100.10</td>
</tr>
<tr>
<td>3.046</td>
<td>0, 1.015, 2.031, 3.046, 4.061</td>
<td>3.049 0.020 0.3982 0.1306 0.9999</td>
</tr>
</tbody>
</table>

\(^a\) Coefficient of correlation.
\(^b\) Mean for five independent analyses.
The robustness of the proposed method was established by deliberately changing the volume of $2.205 \times 10^{-3}$ M cefixime, 1.8 mL ($\pm 0.2$ mL) for the determination of Cu(II). The synthetic mixture sample solution containing 5.0 $\mu$g mL$^{-1}$ Cu(II) was analyzed five times repeatedly by the proposed method. Percentage recovery and RSD were found to be 99.84% and 0.15%, respectively, indicating robustness of the proposed method.

Under the optimized experimental conditions, the calibration graph by considering 7 independent concentration levels of Cu(II) was constructed by plotting the absorbance against initial concentration of Cu(II) (Fig. 7). Beer’s law is obeyed in the concentration ranges of 1.015-8.122 $\mu$g mL$^{-1}$ with apparent molar absorptivity of 8.29 $\times$ $10^3$ L mol$^{-1}$cm$^{-1}$ and Sandell’s sensitivity (0.008 $\mu$g/cm$^2$/0.001 absorbance unit). Linear regression equation is obtained by statistical treatment of the calibration data ($n = 7$) which is fitted with the straight line equation in the form of $A = a + bC$, where $A$ is absorbance at 336 nm, $C$ is concentration in $\mu$g mL$^{-1}$, $b$ is slope and $a$ is intercept of calibration. High value of correlation coefficient (0.9999) indicated excellent linearity (Table 4). The experimental intercept of the calibration line was tested for significance of deviation from the theoretical intercept, i.e. zero. For this justification, $t$-value calculated from relation, $t = a / S_a$ found to be 1.61, did not exceed the tabulated $t$-value (2.571, $\nu = 5$) at 95% confidence level. This indicated that the intercept in the calibration equation of the proposed method is not significantly different from zero. Thus, the proposed method is free from procedural error.

The applicability of the proposed method for the determination of Cu(II) in synthetic mixture sample has been tested. Results of the proposed method were statistically compared with those of reference method$^{32}$ using point and interval hypothesis tests. The paired $t$- and the $F$-values at 95% confidence level were calculated and found to be less than the tabulated $t$- (2.036 at $\nu = 8$) and $F$- (6.39 at $\nu = 4, 4$) values at 95% confidence level,$^{39}$ thus confirming no significant difference between the performance of the proposed method and the reference performance of the proposed method and the reference method (Table 5). Thus, the bias calculated by interval hypothesis test in the form of lower limit ($\theta_L$) and upper limit ($\theta_U$) were in the range of 0.98-1.02.

The performance of proposed method is also tested in sea and well water samples collected from Muscat, Oman. Results of analysis were found in good agreement with reference method (Table 6). Thus, the proposed method is suitable for routine analysis of Cu(II) in real samples of water. The speed of analysis and less number of reagents utili-
lized in the proposed method are the main advantages of the proposed method as compared to reference method.

CONCLUSIONS

The proposed method is a directUV-spectrophotometric method as compared to reference method (extractive spectrophotometric method). The proposed method is a simple and accurate for the determination of Cu(II) in synthetic mixture and water samples. The method has advantage of using a commonly available solvent i.e. 1,4-dioxan with the use of one reagent, i.e. cefixime. The proposed 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, vegetable and pharmaceutical samples.

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Table 5. Point and interval hypothesis tests for the determination of Cu(II) in synthetic mixture sample for the evaluation of bias of the proposed method with that of the reference method at 95% confidence level

<table>
<thead>
<tr>
<th>Proposed method</th>
<th>Reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Paired t-value</td>
<td>F-value</td>
</tr>
<tr>
<td>$t_0^a$</td>
<td>$F_0^c$</td>
</tr>
<tr>
<td>99.84 0.15</td>
<td>99.91 0.15</td>
</tr>
<tr>
<td>0.721 1.01</td>
<td>0.998 1.003</td>
</tr>
</tbody>
</table>

$^a$ Mean for 5 independent analyses.
$^b$ Theoretical $t (= 8)$ and $F$-values ($= 4, 4$) at 95% confidence level are 2.306 and 6.39, respectively.
$^c$ A bias, based on recovery experiments, of ± 2% is acceptable.

Table 6. Determination of Cu(II) in sea and well water samples by the proposed UV spectrophotometric method and the reference method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of Cu(II) in µg mL$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proposed method</td>
</tr>
<tr>
<td>Sea water</td>
<td>2.164</td>
</tr>
<tr>
<td>Well water</td>
<td>1.022</td>
</tr>
</tbody>
</table>