Cloud Point Extraction as a Procedure of Separation and Pre-Concentration for Cupper (II) Determination Using Spectrofluorometric Techniques

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Abstract:

Cloud point extraction (CPE) is a separation and pre - concentration method that has been considerably applied for trace metal determination in quite a lot of different matrices. Its most important advantages are simple experimental procedures, low cost, high pre - concentration factors, environmental safety and agreement with the "green chemistry" principles. In this method used a TritonX-114 as a surfactant for separation two phases aqueous and organic phases .Spectroflurometric determination then used for determination of Cu(II) at λ_{ex} =366 nm and λ_{em} = 447 nm after enhancement reaction with Amoxicillin drug .The chemical variables affecting the separation and determination procedure were optimized. In optimum conditions Beer' s law was obeyed in the range 0.01–1.1 µg. mL^{-1}. The proposed method was applied successfully to the determination Cu(II) in natural and waste water.

Keywords: Cloud point extraction, metal trace analysis, spectroflurometric techniques ,TritonX-114

INTRODUCTION :

Amoxicillin drug has a chemical formula is known as a (2S,5R,6R)- 6-{[(2R)-2-amino- 2-(4-hydroxyphenyl)-acetyl]amino}- 3,3-dimethyl- 7-oxo- 4-thia- 1-azabicyclo[3.2.0]heptane- 24-carboxylic acid it is used as a ligand for the determination of copper ion by cloud point extraction (CPE), Amoxicillin it has been wide range for the antibiotic that fights bacteria. Amoxicillin is used to treat many different types of infection caused by bacteria, such as tonsillitis, bronchitis, pneumonia, gonorrhea, and infections of the ear, nose, throat, skin, or urinary tract and the chemical structure of the drug is shown in figure 1[1-2] .
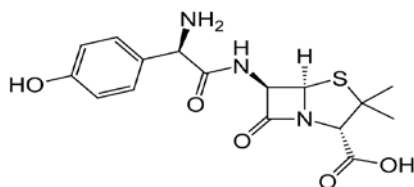

Fig.1. The chemical structure of Amoxicillin

Cu(II) at high concentrations is very toxic because its ion is soluble in water and may be easily absorbed into living organisms. Exposure to the metal occur through air, dust, food/drink cans, drinking water contamination (water inside copper pipes) and gasoline, mainly due to industrial development generated wastes which contain heavy metals to environment. [3] Copper is an essential element for enzymes, but it is also toxic when its concentration is relatively high. This element is needed by many living organisms at only very low levels (nearly 40 ng mL^{-1}) to maintain carbohydrate and lipid metabolism and heart and blood vessel activity. [4-5] Several various method for the determination of Cu(II) ,such as flame atomic absorption . [6-12]Since the past decade, few articles have appeared in the chemical literatures reflect the applications feature of CPE coupled with atomic spectrometers and other techniques for copper extraction and preconcentration from water, and food matrices.

EXPERIMENTAL

Instrumentation

Spectrofluirometer (Shimadzu- Model RF-5301PC) was used for all the measurements in this methods for the determine the concentration of the copper (II) . A digital pH meter (inolab 720 model WTW Germany) was used for all pH measurements. A thermo stated bath Thermo, balance (Circulator England) maintained as the desired temperature was used for the cloud point extraction experiments, a centrifuge(PLC 03 USA),AA-6300 Shimadzu Atomic Absorption spectrophotometer type flame atomic absorption spectrophotometer .

Reagents and solutions

All the chemicals used were of analytical reagent grade, Stock solutions of Cu(II) was prepared by dissolving appropriate amounts of $Cu(NO_3)_2.2H_2O$ in deionized water. The non-ionic surfactant, Triton X-114 was used without further purification. A(0.01) mol L^{-1} solution of Amoxicillin stock solution was prepared by dissolving appropriate amounts (0.1937) gram of this drug in deionizes water completely the volume by deionized water in 50 mL suitable volumetric. The pipettes and vessels used for trace analysis were kept in 5% nitric acid for at least 24 h and subsequently washed three times with distilled water.

Cloud point extraction procedure (CPE)

For the CPE procedure , aliquots of 5 mL of a solution containing of the Cu(II) , Triton X-114 , Amoxicillin and a buffered solution at a suitable pH was kept in the thermostatic bath maintained at 50 ◦C at a period 20 min. Because the surfactant density , was 1.052 gm.L^{-1}, the surfactant-rich phase can settle through the aqueous phase, then the centrifuging for 20 min at 4000 rpm accelerated the phase separation, and then make a cooling in an ice bath, the surfactant rich phase became viscous and was still at the bottom of the centrifuge tube . Simply a syringe centered in the bottom of the its tube can readily discard the aqueous phases. In order to decrease the viscosity and facilitate sample handing prior to the spectrofluirometer instrument ,5 mL of ethanol was added to the surfactant rich phase. The final solution was introduced into the spectrofluorimetry cell to complete its measurement of CPE.

Fluorescence measurements

A spectrofluirimetric method was used to enhancement the Amoxicillin fluorescence spectrum   intensity (Amoxicillin – Cu(II))   for the determination of its metal ion in all the experimental . The fluorescence intensity of its complex was measured in the intensity solution at an excitation wavelength of 366 nm and an emission wavelength of 447 nm and calibration curve was constructed. The fluorescence intensity of its complex is dissolved in ethanol after extraction was measured and the Cu(II) was determined. All measurements were carried out at room temperature after the optimization for the temperature of its

cloud point extraction for its ion, Fig.(2) show the excitation and the emission spectrum of Cu(II) – Amoxicillin complex.
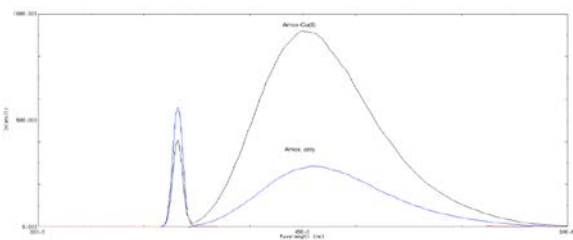

Fig. 2. Excitation and emission enhancement spectrum of Amoxicillin and its Cu(II) complex.

RESULTS AND DISCUSSION

Under the optimum experimental conditions which are represented in the coming results . Cu(II) reacts with Amoxicillin to form hydrophobic complex , which is subsequently trapped in surfactant micelles.

Effect of pH

Cloud point extraction for the copper species was prepared at different buffered pH solutions. The separation of copper ion by cloud point extraction involves prior formation of a complex with sufficient hydrophobic and the Amoxicillin drug to be extracted into the small volume of the surfactant-rich phase. The pH of the solution was optimized for the non-ionic CPE in order to obtain the optimum signal for the selected emission spectrofluorimetry , the pH was adjusted to the desired value by addition of buffer solution . Following the experimental process described in the procedure section it was found that the extraction efficiency is almost dependent on pH conditions for the pH range of (8.5-10) for its complex . (Fig. 3) giving a extraction percentage of its value . This behavior is anticipated due to the hydrophobic, non-ionizable nature of the analytes.
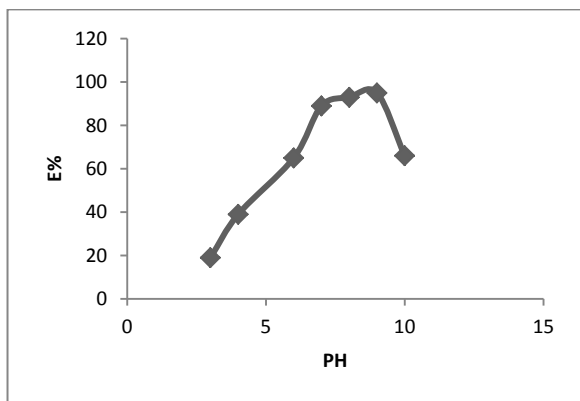

Fig. 3. Effect of pH on the extraction signal and percent of Cu(II) - Amoxicillin complex.

Effect of Amoxicillin concentration on the cloud point extraction

The extraction method via CPE methodology depends on most important step of complexation between organic reagent (drug) and Cu(II), that can be transferred to surfactant after formation cloud point phase .In view of the actuality that the complexation step was also depends on the nature of organic reagent which specify the extraction capability via the formation more stable complexes .For that reason, the effect of drug (Amoxicillin) concentration was carried out by taking 5 mL solution containing 0.5 µg mL^{-1} Cu (II), different amount from (0.2-0.8) mL of

2.0×10^{-4} mol L^{-1} (Amoxicillin), 0.6 mL of 5% TritonX-114 at optimum (pH=9.0) value . Each experiment was and subjected to the CPE process . The results are shown in Figure (4) for extraction percent.
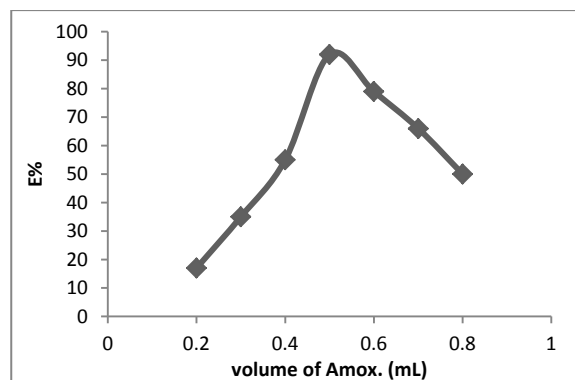

Fig. 4. Effect the volume of Amoxicillin on the extraction percent of Cu(II) - Amoxicillin complex.

Effect the volume of Triton X-114

In which case the amount of TritonX-114 whose utilized in CPE method for the separation and extraction of any metal or organic species was a very critical factor because its factor lead to obtained increase the preconcentration factor and minimum phase volume but this is should be by the maximum extraction efficiency . The effect of TritonX-114 concentration was examined by taken a 5.0 mL volumetric flask including of 0.5 µg.mL^{-1} from Cu(II) and 0.5 mL of 2.0×10^{-4} mol L^{-1} from (Amoxicillin) at the optimization pH value equal to (9.0) and different concentration from a nonionic surfactant TritonX-114 corresponding to the volume from (0.1-0.7) mL from its 5.0 % (v/v) nonionic surfactant. Figure (5) it can be seen that the optimum quantity was extracted at the volume 0.6 mL of 5.0% (v/v) Triton X-114 and thus chosen for further experiments
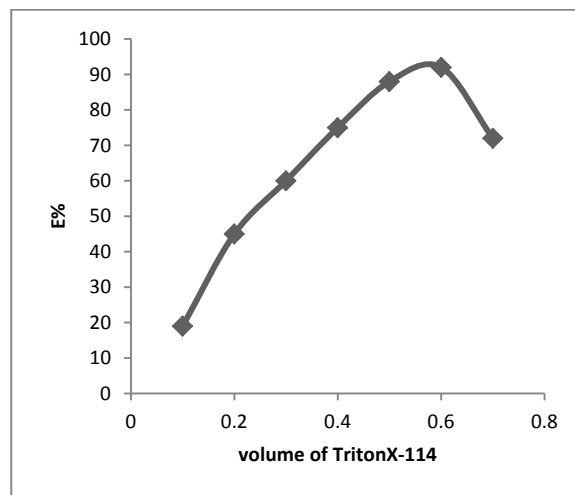

Fig. 5. Effect the volume of TritonX-114 on the extraction percent of Cu(II) - Amoxicillin complex.

Effect of the equilibrium temperature and time

In the method of cloud point extraction system, temperature of extraction is very important parameter for micelle as occurring. The effect of equilibration temperature and time incubation in the CPE system was investigated at 20 to 80 °C. At temperatures lower than 30 °C the separation of the two phases at the cloud point temperature was not complete. After 60 °C, the signal intensity was approximately same value or decrease because its complex will be dissociated at high temperature (Fig. 6) . The

incubation time in the water bath was kept at 20 min. for Cu(II) complex which is sufficient for the completion separation into two phase aqueous and organic phase of these processes.
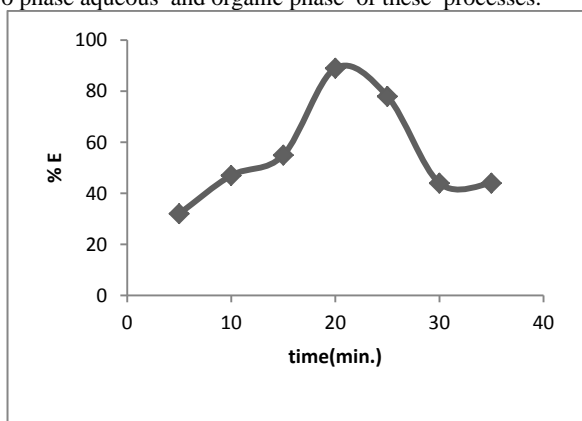

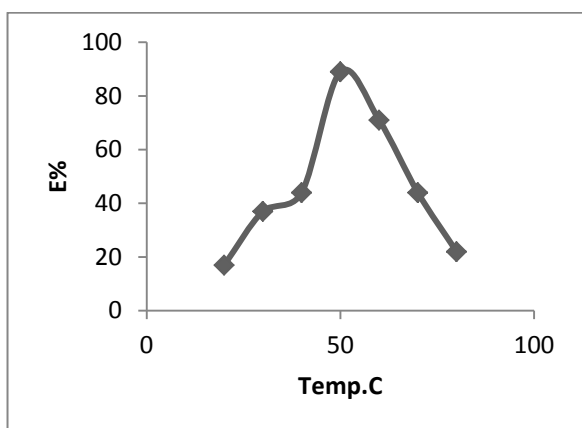**Fig. 6. Effect of time incubation on the extraction percent of Cu(II) - Amoxicillin complex.**

Fig. 7. Effect of temperature on the extraction percent of Cu(II) - Amoxicillin complex.

The effect of the equilibrium temperature was examined by taking 5 mL aqueous phase containing of 0.5 µg mL^{-1} Cu (II) and then keeping other factors at optimum conditions. The temperature was varied from 20 to 80 °C at 20 min in a search of optimum value . It was shown that the highest intensity signals and thereby the highest extraction percent were obtained when the temperature at 50 ℃ achieving quantitative extraction that shown in Figure (7) in above paper .

Determination of stoichiometry and stability constants of the complexes

The stoichiometry and stability constant of the Amoxicillin - copper complex was determined by Benesi–Hildebrand method [13-15].

In the case of a 1 : 1 complex, the following equation is applicable:

$1/ F\text{-}F_{\circ} =1/(F\infty\text{-}F_{\circ})K[Me]_{\circ} +1/(F\infty\text{-}F_{\circ})$ (1)

In this line , a graph of $1/F\text{-}F_{\circ}$ versus$1/[Me]_{\circ}$ was constructed where F is the observed fluorescence at each concentration tested , $F_{\circ}$ is the fluorescence intensity of analyte in the absence or without found of metal ion, and$[Me]_{\circ}$ is the concentration of metal ion(copper) . A linear plot is required for this double reciprocal plot in order to conclude 1 : 1 stoichiometry. While when the case where 2 : 1 stoichiometry is predominant, the applicable equation is become and applied is :

$1/ F\text{-}F_{\circ} =1/(F\infty\text{-}F_{\circ})K[Me]^{2}_{\circ} +1/(F\infty\text{-}F_{\circ})$ (2)

Also when the stoichiometry of the complex is 2 : 1 complex ,by using the continuous variation method[15] , a straight line would

be obtained when $1/(F\text{-}F_{\circ})$ is plotted against $1/[Me]^2_{\circ}$. To calculate the stability constant of the complex, an aliquot of the Amoxicillin to give a final concentration of $1.0 \times 10^{3-}$ mol L^{-1} was placed in a 5 mL calibrated flask and different amounts of metal were added to give a final concentration between 0.3×10^{-6}– 8.0×10^{-6} mol L^{-1}. After the addition of 1 mL of buffer solution and optimum volume of TritonX-114 to the solutions and final diluted to volume with distilled water and made a cloud point extraction to make a two phases ,at last the organic phase contain a small amount of the surfactant rich phase is obtained and the fluorescence intensity was measured at $\lambda_{em.}$=447 nm, and the stability constant is determined by dividing the intercept on the slope as a visible emission[16-17] ,this method is made at 50 ,60 C° because this degree gives a best extraction of the complex ,as shown in fig.(8) that a pure the Cu(II) – Amoxicillin is 1 : 2 .
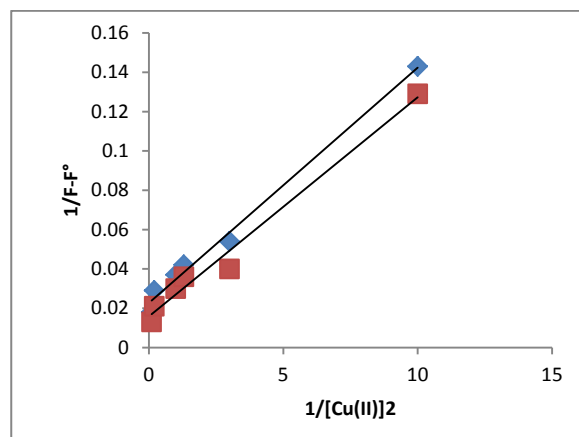

Fig. 8. Benesi-Hildebrand s plots for 1:2 Amoxicillin - copper complex .

Calibration Graph for its complex

After complete all the optimization conditions to obtained a high sensitivity and low detection limit for the determination of Cu(II) in different samples therefore , a linear calibration curve were obtained by plotting the intensity signal against the concentrations of Cu(II). The calibration information are shown in Figure (9) and the sum of analytical parameters are summarized in table (2). The following regression equation was obtained: y = (249.85 x +9.2734) , and r = 0.9958 and n = 10,where y is the intensity, and x is the copper (II) concentration in ng mL^{-1}. This regression line had a coefficient of determination (R^2) of 99.58% which suggests it is statistically valid. Analysis of variance (ANOVA) for the regression line was also carried out as shown in Table (1). ANOVA analysis supports that there is a strong significant relationship between the concentration of the analyte and intensity units as F critical, $F_{1, 9}$=5.117 << 2170.609
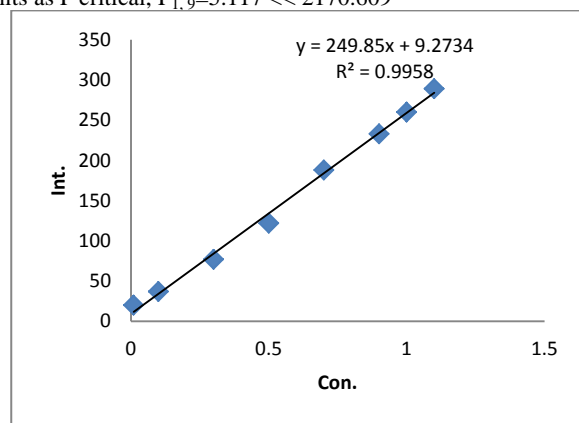

Fig. 9. Calibration graph , Cu(II) - Amoxicillin complex.

Table 1 Analysis of Variance of regression line of Cu(II)

Regression statistics					
Multiple R	0.9958				
R square	99.58%				
Adjusted R square	0.995				
Standard error	0.0014				
Observations	10				
ANOVA					
Model	df	SS	MS	F	Significance F
Regression	1	0.008	0.008	2170.609	0.000
Residual	8	0.000	0.000		
Total	9	0.008			
Coefficients		Standard error	t stat	P-value	
0.998		0.001	1.086	0.000	
	Lower 95%	Upper 95%	Co linearity statistics		
Constant Intensity	-0.002	0.004	1.000		

Table 2 Method validation of the spectroflurimetric determination of Cu (II) using CPE procedure.

Parameter	Value
Excitation wave length $\lambda_{ex.}$ (nm)	366
Emission wave length $\lambda_{em.}$ (nm)	447
Regression equation with CPE procedure	y=249.85x+9.2734
Correlation coefficient(r)	0.9958
Concentration range (ng mL^{-1})	0.01-1.1
Limit of Detection (ng mL^{-1})	0.005
Limit of Quantitation (ng mL^{-1})	0.059
Composition of complex (M: L)	1:2
RSD% (n=7) at 0.5 µg mL^{-1}	1.34
Preconcentration factor	98
Enrichment factor	113

Precision and Accuracy

Although the determination of copper ion in water samples by proposed method was made by application the standard additions method for the avoided the matrix interferences and to make a high precision , but to ensure that made a seven reproduce analyses of 0.5 µg mL^{-1} copper solution following the general CPE system gave repeatability in term of relative standard deviation (RSD) of 1.34% (table 2). For the reason that the commercial certified reference material for water samples was not available and in order to investigate if the proposed method is subjected to systematic error, the accuracy in term of recovery percent was studied by spiking of 0.1, 0.2 and 0.4 µg mL^{-1} to an appropriate amount of standard copper(II) solution and then the same steps were followed with the general CPE procedure. The results were tabulated in table (3), indicating that there is no highly important systematic error and can be able to application this method for the determination of its ion by high accuracy.

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Table 3 Accuracy of the proposed method.

Amount metal ion taken (ng mL^{-1})	Amount metal ion found (ng mL^{-1})	Rec (%)	E_{rel} (%)	Mean Rec% ±t.s/√n
0.1	0.11	98.50	-1.50	
0.2	0.23	97.54	-2.46	95.37±4.63
0.4	0.45	95.50	4.5-	

Thermodynamic Parameters

The dependence of the stability constant value on the temperature, a thermodynamic methods was considered to be responsible for this interaction. The thermodynamic parameters: enthalpy changes(ΔH), entropy changes(ΔS), and free energy changes(ΔG) are the main evidences to determine the binding mode. The free energy change (ΔG) can be estimated from the following equation, based on the binding constant at different temperatures.

$$\Delta G = -2.303RT \log K \qquad (3)$$

where R is the gas constant, T is the experimental temperature, K is the stability constant at the corresponding temperature. From the value of stability constant at different temperatures, the enthalpy changes can be calculated by using equation (4):

$$\log K_2/ K_1 = 2.303R \ \Delta H \ [1/ T_1 - 1 /T_2] \qquad (4)$$

The entropy changes can be calculated by using equation (5):

$$\Delta G = \Delta H - T \Delta S. \qquad (5)$$

The stability constant of As(III) complex and other thermodynamic parameter are shown in table (4) [18] .

Table 4. Stability constant and other thermodynamic parameter

Metal -complex	Log K		-ΔG,KJ/mol		ΔH,KJ/mol	ΔS,KJ/mol K	
	50 °C	60 °C	50 °C	60 °C		50 °C	60 °C
Cu $^{2+}$	9.61	9.79	58.23	60.78	38.21	0.435	0.435

From the results in table (4) the ΔH indicating that the extraction reactions are endothermic and the negative values of ΔG dictates that the solubilization are spontaneous phenomena.

Determination of Cu(II) in water sample

After calculated all the above parameter for found the optimal conditions , this proposed method was applied to the determination of copper (II) ion at pH=9.0 in the seven water samples .For negligible the interferences effect that found in water samples , this determination of its ion was applied by using standard additions method to ensure that determination of trace level of its ion without the other ions that will be found in its samples and using a masking agent of 0.4 mL from a mixture from 0.01 mol L^{-1} citric acid and 5-sulphosalicylic acid for approximately all ions. The results of the proposed method (CPE – spectroflurimetric) were compared with flame atomic absorption spectrophotometer method in our laboratory it has also been illustrated in Table (5).

Table 5 Spectrofluorimetric determination of Cu(II) in water samples

NO.	water samples*	Concentration of Cu(µg mL^{-1})	
		Introduce method [a]±SD	Flame-AAS [b] ±SD
1.	water1	0.467±0.0026	0.488±0.0027
2.	Water2	0.521±0.0013	0.568±0.0042
3.	R.O. water1	0.221±0.0032	0.289±0.0050
4.	R.O water2	0.561±0.0043	0.601±0.0062
5.	River water1	0.342±0.0018	0.398±0.0024
6.	River water2	0.385±0.0029	0.412±0.0012
7.	River water3	0.462±0.0062	0.521±0.0032

*from Al-Hilla city, Babylon Governorate
[a] mean ± standard deviation; n = 5
[b] mean ± standard deviation; n = 3

The student t-test was applied to evaluate the significance level of results obtained between the proposed method and the sensitive flame atomic absorption spectrophotometer FAAS for further checking of the applicability and reliability of CPE-spectroflurimetric method. The statistical analysis of the results shown in Table 5 and figure 10 proved that there was no significant difference at 95% confidence level for the determination of Cu(II) by both methods. The calculated

experimental values $\lvert t \rvert$ was 0.092 for Cu(II) results. This value is less than the critical $t_{0.05,12} = 2.18$(two-tailed) indicating that no difference between the two means (Ho, $\mu_1 = \mu_2$) copper to the same statistical conclusion. For the assessment, the precision of the measurements by both methods, F-test analysis provided evidence that the variability in the proposed method and FAAS were not significantly different at 95% confidence level as the test statistic F of 1.22 in Cu measurements obtained by both methods were less than the critical $F_{0.05, 6,6} = 5.43$ two-tailed.

CONCLUSIONS

A simple spectrofluorimetric process for the determination of copper (II) was developed. Optimization of the parameters produced a stable and highly fluorescence intensity for its ion – Amoxicillin complex. This methodology resulted in a low value of LOD and LOQ its shown in above table for its ion. Simple and rapid method by sensitive value of spectrofluorimetric determination of its ion in water sample.

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