

Cloud Point Extraction and Spectrophotometric Determination of Trace Copper(II) Using Amino Acid Methionine As a Complexing Agent

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Abstract

Cloud point extraction (CPE) is a separation technique on the basis of phase separation properties of non-ionic surfactant above the cloud point temperature and the solubilization phenomena of surfactant rich phase. Cloud point extraction exhibits many attractive advantage such as simplicity, low lost, high enrichment factor, non flammable, non volatile and less toxic surfactant, moderate extraction conditions and friendliness to the environment. A method passed on the cloud point extraction (CPE) for the trace analysis of Cu (II) in water samples is described in this study. The analyte in the initial aqueous solution are complexed with methionine at pH=8 and 5mL of 10% (v/v) Triton x-100 is added as surfactant. Following phase separation at (85°C) based on the cloud point of the mixture and dilution of the surfactant-rich phase with acidified methanolic solution. The enriched analyte are determined by Uv-visible spectrophotometry. After optimization of the complexation and extraction conditions, the preconcentration and enrichment factor of Cu (II) was found to be 90,89 respectively. Under optimum conditions like pH, methionine concentration, surfactant concentration, operation temperature and electrolyte effect, calibration graph showed linear trend in range of (0.05-4) $\mu\text{g/mL}$ with molar absorptivity (19621.5) $\text{L}\cdot\text{mol}^{-1}\text{cm}^{-1}$ Sandell's sensitivity and detection limit of 0.0032 $\mu\text{g}\cdot\text{cm}^{-2}$, 0.0071 $\mu\text{g/mL}$ respectively for copper ion.

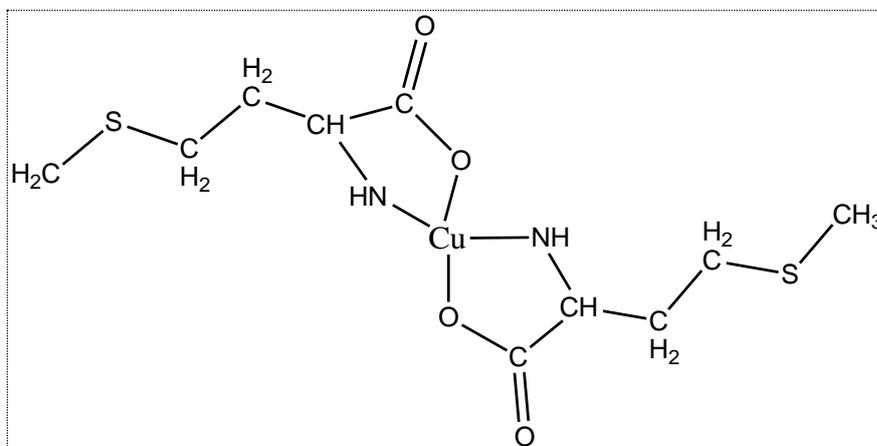
Key words: Cloud Point Extraction, Spectrophotometry, Copper, TritonX-100

INTRODUCTION

Copper is nutritionally essential metal and widely distributed in nature. It's occurs in it's nature state of which are those containing sulfide compound but also those with oxides and carbonate copper is widely used in electrical wiring roofing, various alloys, pigment, cooking utensils, piping and in chemical industry. Copper salts are uses in water supply system to control biological growth in reservoirs and distribution pipes and to catalyze the oxidation of manages. Copper forms a number of complexes in natural waters with inorganic and organic ligands. Among the comment aqueous species are Cu^{+2} , $\text{Cu}(\text{OH})_2$ and CuHCO_3^+ . Corrosion of copper containing alloys in pipe fitting, may introduce measurable amount of copper into the water in pipe system copper is considered an essential trace element for plants and animals some compound are toxic by ingestion or inhalation⁽¹⁾. The important of metal ions for smooth operation of plant and animal organism was evidenced to synthesis and characterization of biological compound containing metal ions, due to their applicability in pharmacy, medicine, agronomy and nutrition. Amino acid complex with metals are similar in structure to the natural ones present in the body and release trace elements is exactly the cell or tissue that needs them⁽²⁾. Methionine is one of the amino acid containing sulfur, it help to prevent disorders of the hair, skin and nails in lowering the cholesterol levels by increasing the liver's production of lecithin and reduce fat build-up in the liver and body⁽³⁾. Methionine is a biological chelating agent may lower the degree of toxicity for the formation of chelate with toxic metals⁽⁴⁾. There is a great need to develop a simple, sensitive, selective and in expensive method for the determination and continuous monitoring of Cu levels in environmental and biological specimens⁽⁵⁾. Spectroscopy has played a vital role in the development of modern atomic theory. In addition petrochemical method have provided perhaps the most widely use tools for the elucidation of molecular structure as well as the quantitative and qualitative determination of inorganic and organic compound⁽⁶⁾. Spectrophotometric method is remarkable for their versatility, sensitivity and precision. Almost are can be used for all elements, expect for the noble gases. A very extensive range of concentration may be covered, from macro quantities to traces (10^{-8} – 10^{-6} %) after preconcentration/ Spectrophotometric method are among most prices instrumental method of analyses⁽⁷⁾. Major difficult involved in determination of trace metals by Spectrophotometric analysis are the lack of selectivity and sensitivity, requiring separation and preconcentration of the samples to be analyzed.

The classical liquid-liquid extraction and separation methods are usually time consuming, labor extensive and require relatively large volumes of high parity solvents. In addition there is concern regarding the disposal of the solvent's used cloud point extraction (CPE) in an attracted. Considerable attention menially because it complies with the "Green chemistry" principle ⁽⁸⁾, as the amount of organic solvent is much less than that of traditional liquid extraction. Moreover, it is simple, cheap, highly efficient, fast, and of lower toxicity than those extraction that organic solvents. This method has been applied for extraction and pre-concentration of some metal ions such as Cu in water samples ⁽⁹⁾ and in environmental samples ⁽¹⁰⁾ this method is based on the property that an aqueous solution of surfactants forms micelles and becomes turbid above a temperature defined as cloud point temperature. Above the cloud point temperature, the original surfactant solution separates into a small volume of surfactant rich phase and a bulk of diluted aqueous phase, in which the concentration of surfactant is close to the critical micellar concentration (CMC). Any analyte solubilize in the hydrophobic core of the micelles will be concentrated into the small volume of the surfactant rich phase, which can subsequently be determined by different Spectrophotometric techniques. ^{(11) (12) (13)}

The present work report the results obtained in a study of the cloud point extraction and pre-concentration of Cu(II) after the formation of it's complex with methionine (Schem1). Triton X-100 was used as a non- ionic surfactant for CPE following by Spectrophotometric analysis extracted phase. The factors influencing the efficiency of Spectrophotometric determination of Cu (II), by the proposed method were systematically studied.



Scheme (1) The probable chemical structure of Cu (II) with methionine

Experimental

Apparatus

- 1- A Shimadzu 1650 PC model double-beam UV-Vis spectrophotometer (Japan) working at wavelength of 190-1100 nm (± 0.3 accuracy and ± 0.1 repeatability)
- 2- A Microprocessor pH meter 211 model (Triup International Corp, Italy) pH –Meter.
- 3- Centrifuge: REMI centrifuge, Equipment, Bombay-India, 5000rpm.
- 4- Water bath WB 710 model (OPTIMA, Japan).

Material and Reagent

All chemicals used were of analytical reagent grade or chemically pure grad and double distill of water was used for all dilution of reagent and samples.

- 1- Standard Cu^{+2} ($1000 \mu\text{g ml}^{-1}$) stock solution of copper was prepared by dissolving (0.389gm) $\text{CuNO}_3 \cdot 6\text{H}_2\text{O}$ in distilled water and diluting to the mark in 100ml volumetric flask. The working standard solutions were prepared freshly by appropriate diluting of the stock solution
- 2- (10% v/v) Triton x-100
10 ml of Triton x-100 was added to 100ml volumetric flask and diluted up to the mark by distilled water.
- 3- Methionine as a reagent purity (98.5%) fluke a solution of (5×10^{-4}) M was prepared by dissolving 0.007gm in 100ml volumetric flask and the diluted up to the mark by distilled water
- 4- Buffer solution (pH8) was prepared by mixing 100ml of 0.025 M di sodiumtetraborate (borax) with 41 ml of 0.1 M HCl.

General procedure of CPE:

For the cloud point extraction, to the aliquots of the standard solution, 0.5ml Triton x-100 (10% v/v), 0.7mL methionine (5×10^{-4}) M and 2mL of Borax buffer solution were added and the volumes were made up to 5mL with distilled water. The mixtures were kept for 30min in the thermo stated bath at 85°C . Separation of the two phases was accomplished by centrifugation for 4min at 4000rpm. The aqueous phase was easily decanted by simply inverting the tube.

The surfactant- rich phase obtained by this procedure was dissolved with acidified 3 ml methanolic solution. This solution transferred to 2mL quartz cell. A blank solution was prepared in the same way.

Result and Discussion

The optimization of the method was carefully studied in order to achieve complete reaction formation, highest sensitivity and maximum

absorbance spectral characteristics of Cu (II) – methionine complex under the conditions used. Cu (II) ion and methionine reagent form a complex in a surfactant rich phase after CPE procedure against reagent blank. The complex of Cu (II) with methionine has a maximum absorption at 294 nm at (pH=8) against reagent blank (Fig.1-1).

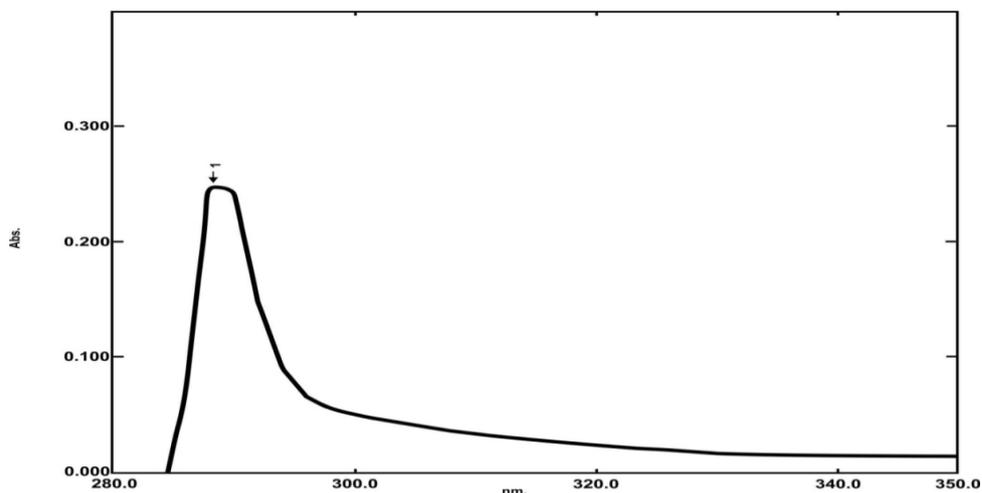


Fig. (1-1a) The absorption spectra of methionine in methanol (conditions of measurement: 0.7mL of 5×10^{-4} M methionine; 0.5mL of 10% v/v Triton x-100, 2mL of borax buffer solution at pH= 8).

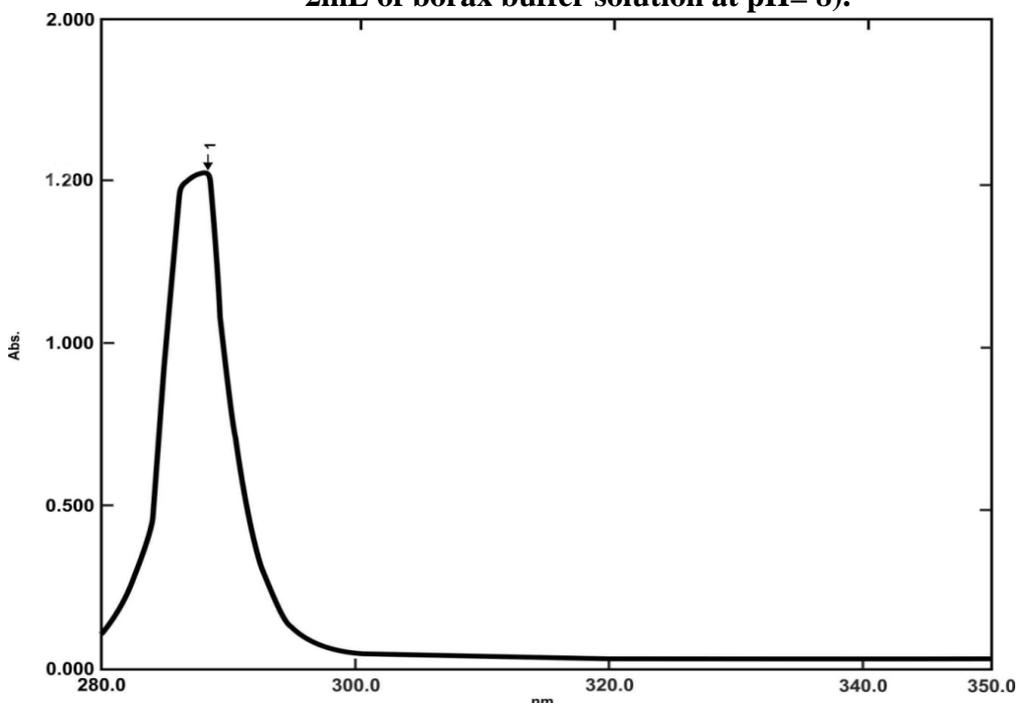


Fig. (1-1b) Absorption spectra of copper complex with methionine in methanol (conditions of measurement: 4 μ g/mL of Cu(II); 0.7mL of 5×10^{-4} M methionine, 0.5mL of 10% v/v Triton x-100; 2mL of borax buffer solution at pH= 8 against blank reagent).

Optimization parameter for CPE of Cu(II).

All of the parameters in the CPE method such as PH, surfactant, ligand concentration, heating time and temperature, centrifuge time and the effect of interference ions for extraction processes were optimized by one factor a time (OFAT) procedure to improve the sensitivity and detection limit for determination of Cu(II).

Effect of pH on CPE:

The extraction of metal ions by the CPE method involves the formation of a metal chelate with sufficient hydrophobicity to be extracted into the small volume of the surfactant- rich phase. The pH plays a unique role in metal- chelate formation and in subsequent cloud point extraction yield. Therefore, pH was the first parameter evaluated for its effect on the determination of copper ions. Figure(1-2) shows the effect of pH in the range of (3.5–9.0) on the extraction of the Cu(II) complex. Quantitative extraction efficiency was achieved at pH 8.0. For this reason, pH 8.0 was used for the subsequent work. The decrease in absorbance at Ph greater than 8 is due to precipitate of copper as black oxide form.

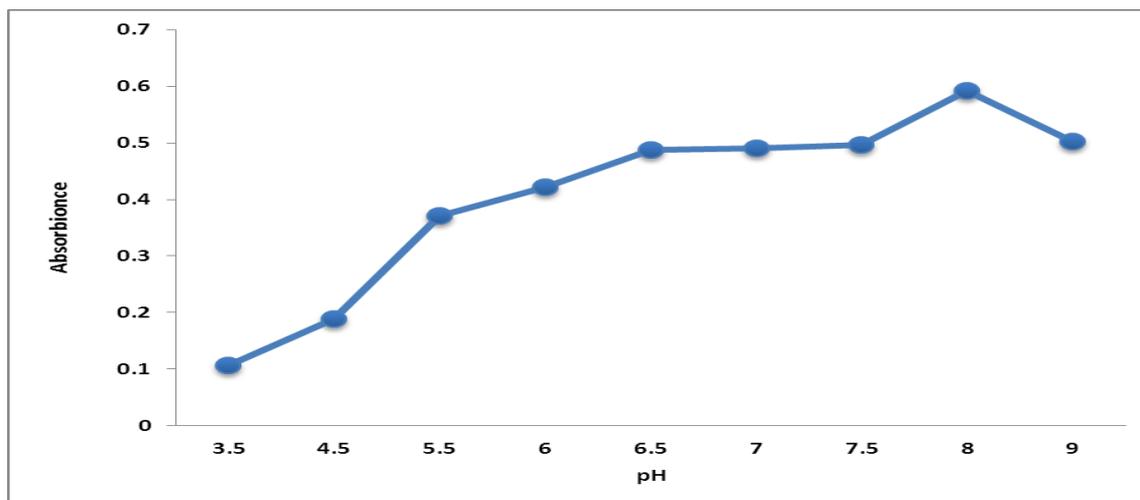


Fig. (1-2): The effect of pH on the absorbance of the Cu (II) – methionine complex at 294 nm. Conditions: 2 µg/mL Cu (II), 0.5 mL of 10% Triton X-100, 0.7mL of 5×10^4 M methionine

The Effect of the methionine concentration on the CPE of Cu (II) was investigated by measuring the absorbance signal according to the CPE solution containing 2µg/mL Cu (II) and various amounts (0.2-1.4)mL of 5×10^4 M of the methionine ligand. The absorbance increase rapidly as the concentration of ligand increases and decreases slightly with further increase in the chelating agent, reaching the optimum value, which is

considered as a complete extraction. A concentration of 0.7mL of 5×10^{-4} M was chosen as the optimum concentration as shown Figure(1-3)

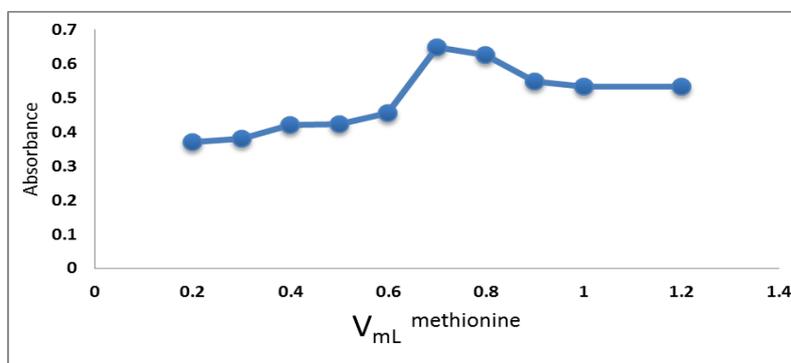


Fig.(1-3): The effect of methionine concentration on the absorbance of the Cu (II) – methionine complex at 294 nm. Conditions: 2 μ g/mL, 0.5 mL of 10% Triton X-100, PH=8.

Effect of Triton x-100 concentration

The non ionic surfactant, Triton x-100, was chosen because of its commercial availability in a high purified homogeneous form, low toxicological properties and cost also, the high density of the surfactant rich phase facilitates phase separation by centrifugation Triton x-100 permits use in the extraction and/or pre-concentration of a large number of molecules and chelates^(14,15). Different volumes of Triton X-100 (10% v/v) ranging from (0.1- 1.2) mL were used in this study at previously optimum conditions. As shown in Figure 4 , the absorbance for ion increased by increasing the Triton X-100 concentration up to 0.5 mL of 10% (v/v) for Cu(II) , and then the absorbance was slightly low because the assemblies may be insufficient to quantitatively entrap the hydrophobic complex⁽¹⁶⁾. Therefore, 0.5 mL of 10% (v/v) Triton X-100 was used as the optimum concentration for copper ion.

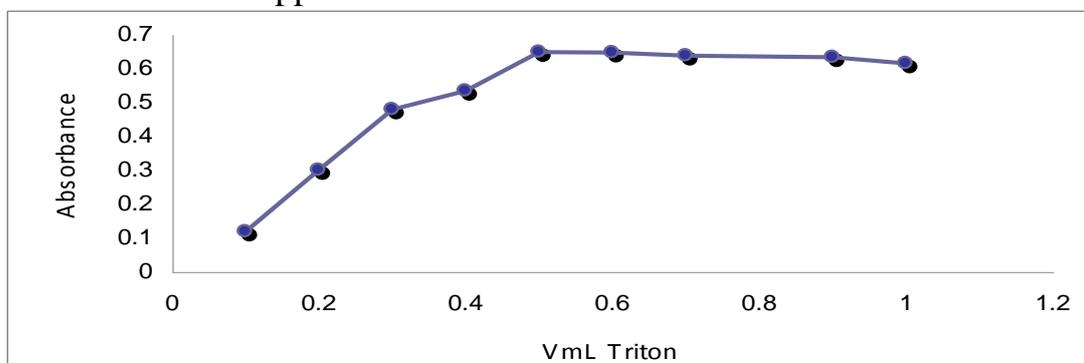


Fig. (1-4): The effect of Triton X-100 concentration on the absorbance of the Cu (II) – methionine complex at 294 nm. Conditions: 2 μ g/mL, PH= 8, 0.7mL of 5×10^{-4} M methionine

Effects of Equilibration Temperature and Time

It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature, which compromise completion of reaction and efficient separation of phases. It was found that a temperature which compromise completion of reaction and efficient separation of phases. It was found that a temperature of 85°C is adequate Cu analysis. The dependence of absorbance upon equilibration time was studied within a rang of (20-60) min. An equilibration time of 30 min was chosen as the best to obtain quantitative extraction.

Effect of centrifugation time:

The effect of centrifugation time on the extraction efficiency was also studied within a range of (1-10)min, a centrifugation time of 4min at 4000 rpm was selected, since the analyte extraction in this time is almost quantitative and the phase separation occurred at the end of this period and no considerable enhancement was observed with longer time.

Effect of ionic strength:

The effect of the ionic strength on the phase separation of anionic surfactant Triton x-100 from water was examined by addition of sodium chloride up to salt saturation conditions. The result indicate no effect on the extraction, this result is possibly due to complete of micelle aggregation through equilibration time and equilibration temperature. This is in agreement with the result reported in the literature, which demonstrate that an increase in the ionic strength in micelle mediated systems dose not seriously alter the extraction efficiency of the analyte⁽¹⁷⁾.

Analytical data and calibration graph:-

Under the optimum experimental conditions by CPE procedure for the determination of Cu (II), calibration curve Figure. (1-5) for determination of Cu(II) at 294 were obtained by plotting absorbance versus concentration of Cu(II), 0.5 mL of 10% Triton X-100 in medium buffered at pH= 8 final solution was introduced into spectrophotometer. In this case, the calibration curve using the preconcentration system for copper ion was linear with a correlation coefficient of (0.998). Regression equation was ($Y=0.309X+0.001$).

The limit of detection obtained for determination copper ion by proposed method was 0.0071 µg/mL . The enrichment factor which is defined as the ratio of the slope of calibration curves obtained with and without CPE found to be 89.7 for copper ion. This revealed that the CPE study gave satisfactory analytical figures of merit, and better than other studies⁽¹⁸⁾

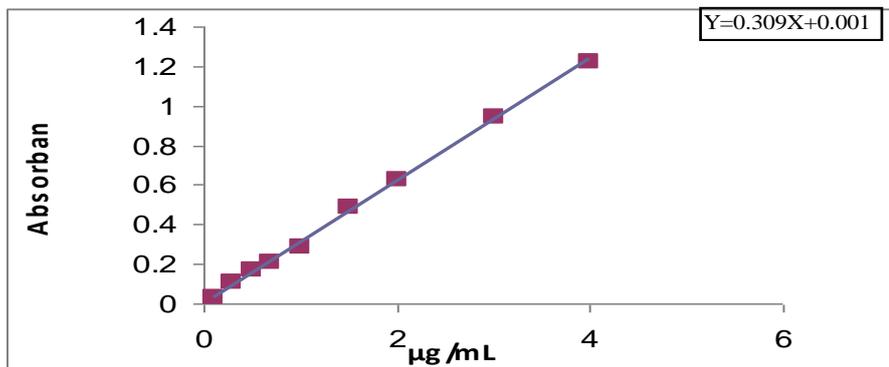


Fig. (1-5): Calibration curve for the determination of Cu (II) under optimum condition.

Table (1-1) Method Validation of the Spectrophotometer Determination of Cu(II) using CPE Procedure with Methionine.

Parameter	Value
λ max (nm)	294
Linearity range ($\mu\text{g}.\text{mL}^{-1}$)	0.1-4
Regression equation	$Y=0.309*x (\mu\text{g}/\text{mL}) +0.001$
Correlation coefficient (r)	0.998
Molar absorptivity ($\text{L}.\text{mol}^{-1}.\text{cm}^{-1}$)	$\epsilon =19621.5$
Sandell's sensitivity ($\mu\text{g}.\text{cm}^{-2}$)	0.0032
Detection limit ($\mu\text{g}.\text{mL}^{-1}$)	0.0071
Quantification limit ($\mu\text{g}.\text{mL}^{-1}$)	0.0215
Composition of complex (M:L)	1:2
Pre-concentration factor	90
Enrichment factor	89.7

Effect of Interfering ions

Under the optimized condition, determined for this study the relative error percent of $2\mu\text{g}/\text{ml}$ of Cu(II) ion were studied in the presence of different concentration of various cations and the same general CPE procedure was performed. It is agreed that an extraneous ion deemed to interfere seriously when it gives a relative error percent of more than $\pm 5\%$. It was shown that all the monovalent ions (M^+) have no effect on analytical response or percent recovery, while the divalent ion (M^{+2}) and trivalent ions (M^{+3}) have exceeded the allowable limits of interferences on Cu (II) ion absorbance signal as show in Table (1-2).

Table (1-2): Effect of Divers Ions on the Absorbance Signal of Cu(II) (2µg/ml) by CPE-spectrophotometer

Ions	Conc. µg/mL	Relative error %
K ⁺¹	2	-1%
	20	2%
NH ₄ ⁺¹	2	3.4%
	20	2.7%
Ca ⁺²	2	- 1.7%
	20	- 13.05%
Ni ⁺²	2	40.5%
	20	25.2%
Cr ⁺³	2	37%
	20	18%
Al ⁺³	2	-53.6%
	20	48%

Stoichiometry of the Complexes

The stoichiometry of Cu(II) complex with methionine was determined by continuous variation at pH8. The result shown in Fig.(1-6) show that the stoichiometry ratio between Cu (II) with methionine is 1:2 and this result was agree with the literature⁽¹⁹⁾⁽²⁰⁾.

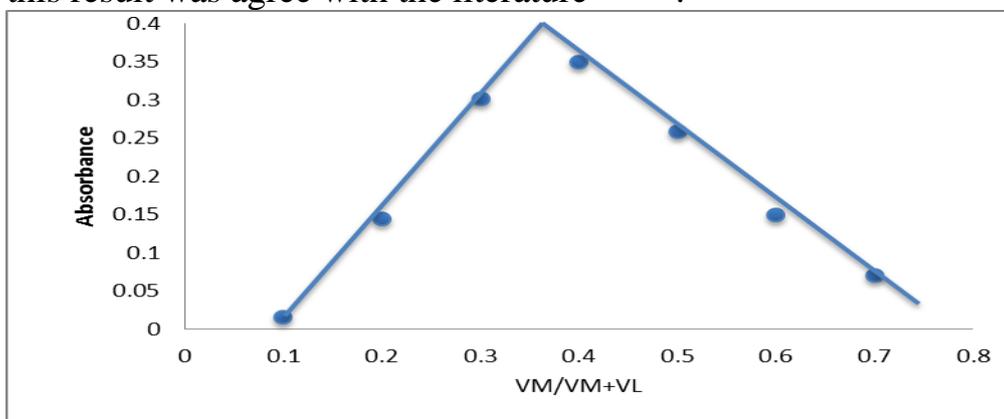


Fig.(1-6).Job's plot of complex between Cu(II)and methionine buffer at pH 8.

Application in Synthetic Sample

The proposed method has been applied to the determination of Cu in various water samples. The reliability of method was checked by recovery experiments. As can be seen from Table1- 3, in all samples, the copper recovery is almost quantitative (93.2-100 %), which demonstrates the applicability of the proposed method for the sample type examined.

Table1- 3: Application of the CPE method on Cu Concentration Measurements in Synthetic Sample.

Real Sample	Taken Conc. $\mu\text{g.mL}^{-1}$	found Conc. $\mu\text{g.mL}^{-1}$	Recovery %
Rain water	0.5	0.498	100
	1	0.932	93.2
	2	1.87	93.5
Tab water	0.5	0.485	97
	1	0.948	94.8
	2	1.907	95.3

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