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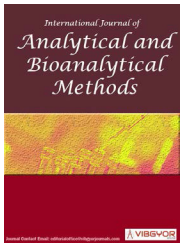
Utilization of Aluminon for Determination of Ultra-trace Levels of Copper in Environmental, Biological, and Water Samples via Cloud Point Extraction Coupled with Spectrophotometry



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Abstract

The present paper outlines novel, simple and sensitive method for the determination of copper using spectrophotometry after separation and preconcentration by cloud point extraction. The cloud point methodology was successfully applied for copper determination after complexation with aluminon (triammonium salt of aurintricarboxylic acid), at pH 3.5. Copper(II) ions was quantitatively recovered in Triton X-114 after centrifugation. Acetonitrile (0.2 ml) was added to the surfactant-rich phase prior to its analysis at λ_{max} 538 nm. The influence of analytical factors including complexing agent, buffer solutions, Triton X-114, temperature, heating time, centrifuge rate and time were examined and optimized. The analytical characteristics of the method (e.g. linear range, Ringbom concentration ranges, molar absorptivity, Sandell sensitivity, optimum limits of detection and quantification, enrichment factor, and improvement factors) were evaluated. Linearity was obeyed in the range of 7.5-220 ng/ml of Cu^{2+} ion. The detection and quantification limits of the procedure were 2.2 and 7.4 ng/ml of Cu^{2+} ion. The tolerance limits of some anions and cations were also examined. The procedure was applied to detect copper in environmental, water and biological samples.

Keywords

Copper determination, Cloud point extraction, Triton X-114, Complexation, Colorimetry, Environmental and biological analysis

Introduction

In biological materials, particularly biological fluids, might be considered a difficult analytical task, mostly because the complexity of the matrix and the low concentration of these elements, which requires sensitive instrumental techniques and often a preconcentration step. Monitoring the presence of toxic trace elements in biological fluids is an extremely essential task to establish occupational and environmental exposure [1-3]. Copper is an important micronutrient and plays an essential role in lipid metabolism and carbohydrate, while at high levels it is an environmental pollutant [4-6].

Copper plays a vital role in the biological systems. In biological systems, copper counter acts the toxicity of zinc suggesting Cu\\Zn antagonism

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[7]. In general, a daily copper intake of 1.5-2.0 mg is essential. However, copper becomes toxic to humans if a large amount is ingested and accumulated in the tissue. For example, because of its mobilization and redox activity, catalytic copper is believed to play an important role in the formation of reactive oxygen species (ROS), such as superoxide anion (O_2^-) and OH radicals. These radicals bind very quickly to DNA and create damage by breaking the DNA strands or modifying the bases and/or deoxyribose, leading to carcinogenesis [8]. Overexposure to copper causes ptyalism, nausea, vomiting, epigastric burning, and diarrhea. High doses of copper result in a series of systematic toxic effects such as hemolysis, hepatic neurosis, gastrointestinal bleeding, oliguria azotemia, hemoglobinuria, hematuria, proteinuria, hypertension, tachycardia, convulsions, and coma [9]. Humans are commonly exposed to copper from drinking water, breathing air, foods or through having skin contact with copper, particles attached to copper or copper-containing compounds [10].

The determination of ultra-trace copper in biological samples is particularly difficult because of the matrix complexity and the usually low concentration of copper, which needs high sensitive instrumental techniques and frequently a preconcentration step [11-13]. However, an excessive uptake of Cu^{2+} can cause serious health problems, including ischemic heart disease, kidney disease, neurodegenerative disease, anemia and bone disorders [14]. Due to their toxicity, the maximum contamination value of Cu^{2+} in the environment and in food was set by several organizations throughout the world to protect human health. For example, the United States Environmental Protection Agency (USEPA) issued the maximum contamination of Cu^{2+} in drinking water at 1.30 mg/L [15]. In Thailand, the pollutant control organization permitted a Cu^{2+} level of 2.00 mg/L in surface water [16]. In addition, the concentration limit of Cu^{2+} for exposure from foods is in the range of 1.2-4.2 mg Cu^{2+}/day as set by the European Food Safety Authority (EFSA) [17]. Therefore, the monitoring of ultra-trace levels Cu^{2+} contaminants in water, food and the environment is necessary.

Recently, various nanocomposites were used as sonochemical fabrication and demonstration as a novel compoundes for removal of organic and inorganic materials [18-22]. A variety of analytical advanced procedures requiring high priced instrumentation are recommended to be dealing with the detection of Cu^{2+} from different matrices at trace amount. These procedures include: Flame atomic absorption spectrometry (FAAS) [23], graphite furnace atomic absorption spectrophotometry (GF-AAS) [24], inductively coupled plasma emission spectrophotometry (ICP-ES) [25]. Although these analytical techniques are highly sensitive and selective, they are quite expensive for the developing countries and need well qualified laboratory personnel and superior laboratory equipment. Moreover, they might not be always available in all routine analytical laboratories. Various of the recommended procedures have some short comings like the availability of sophisticated instruments, tedious work, multi-step procedures for pre-concentration of the analyte, time-consuming operation and low selectivity with higher interference problems. The reported procedures [23] have demerits with respect to their high cost and the instruments used in day-to-day analysis.

Cloud point extraction (CPE) is depended on the phase character of non-ionic surfactants in aqueous solutions, which exhibit phase separation after increasing temperature or addition of a salting-out agent. Separation and preconcentration based on CPE are becoming an essential and practical application of surfactants in analytical chemistry [26,27]. The technique is based on the property of most nonionic surfactants in aqueous solutions to form micelles and to separate into a surfactant-rich phase of a small volume and a diluted aqueous phase when heated to a temperature known as the cloud point temperature. The small volume of the surfactant-rich phase achieved with this technique permits the design of extraction schemes that are cheap, simple, speedy, highly efficient, and of lower toxicity to the environment than those extractions that use the organic solvents. CPE might be an interesting and efficient alternative, once it reduces or eliminates consumption of organic solvents significantly. Trace elements can be extracted to the surfactant-rich phase usually after forming of a hydrophobic complex with an appropriate complexing agent [28]. This approach has been successfully employed to extract and preconcentrate several trace elements from a variety of matrices [28-30] including biological samples [31-34]. Any analyte solubilized in the hydrophobic core of the micelles

will be concentrated into the small volume of the surfactant rich phase which can subsequently be detected by various spectrometric techniques such as FAAS [35], electrothermal atomic absorption spectrometry (ETAAS) [36], inductively coupled plasma mass spectrometry (ICP-MS) [37], inductively coupled plasma optical emission spectrometry (ICPOES) [38], laser induced-thermal lens spectrometry (LITLS) [39], and spectrophotometry [40].

The aim of this article was to extend the use of cloud point pre-concentration strategy to determine copper in various samples for the first time by using triammonium salt of aurintricarboxylic acid (aluminon) as complexing agent and Triton X-114 as surfactant coupled with colorimetry. Triton X-114 was chosen as nonionic surfactant because of its wide use as a cloud point surfactant as well as its low price, commercial availability and lower toxicity.

Material and Methods

Chemicals and reagents

Analytical-grade cupric nitrate, mercuric chloride, sodium molybdate, sodium tungstate, sodium acetate, hydroxylamine hydrochloride and nitrate salts of sodium, potassium, lithium, calcium, barium, beryllium, magnesium, manganese, strontium, cobalt, iron, lead, and nickel (brought from Merck) were of the highest purity available and used without any further purification. Double distilled water was used throughout.

High-purity ethanol, methanol, isopentyl alcohol, chloroform, nitric acid, hydrochloric acid, and ammonia solution (all from Merck) were used without any further purification.

Preparation of cooper and aluminon stock solutions

A standard stock copper solution (1000 mg/L) was prepared by dissolving 0.3802 g of $Cu(NO_3)_2 \cdot 3H_2O$ in double distilled water and diluting to 100 ml in a calibrated 100 ml flask. This solution was standardized by iodimetric titration. Working solution were prepared by appropriate dilution of the stock solution. A 5.0% (v/v) Triton X-114 from E. Merck, Darmstadt, Germany was prepared by dissolving 5.0 ml of Triton X-114 in double distilled water in 100 ml volumetric flask with stirring. Cloud points of Triton X-114 in aqueous solution are 24 °C [41].

A stock solution of 5×10^{-3} M aluminon (achieved by Merck) was prepared by dissolving an appropriate amount in least volume of double distilled water and completed to the mark in 50 mL measuring flask. Acetate, borate, phosphate, thiel and universal buffer solutions of different pH values 2.0-12 were prepared as described early [42]. Stock solutions of 2000 µg/ml of interfering ions were prepared by dissolving appropriate amounts of suitable salts in double distilled water.

Apparatus

All Absorption spectra and absorbance measurements were recorded and measured with a Perkin-Elmer λ3B double beam UV-VIS spectrophotometer with 0.5 mm quartz cuvettes. An Orion research model 601 A/digital ionalyzer fitted with a combined glass-calomel electrode was used for pH adjustment and checking the pH value of buffer solutions. An inductively coupled plasma (ICP) model Varian Liberty 150AX Turbo was used for copper concentration detection. A 30E 148 Sheme fan or Hettich centrifuge was used to accelerate the phase separation process. A water bath with a good temperature control within ± 2.0 °C and a centrifuge with 10 ml calibrated centrifuge tubes (Superior, Germany) were used to achieve and accelerate the phase separation process, respectively.

General procedure

For the cloud point extraction, 5.0 ml of pH 3.5 thiel buffer solution, 4.0 ml of 5×10^{-3} M aluminon solution and 3.0 ml of 5.0% (v/v) Triton X-114 solution, and an aliquot of 10 ml of a solution containing Cu^{2+} ion (0.75-22.0 µg), were kept for 5.0 min in the thermostatic bath at 50 °C and then completed to 100 ml in a 100 ml measuring flask. Separation of two phases was achieved by centrifugation for 5.0 min at 4000 rpm. The mixture was cooled in an ice bath to increase the viscosity of the surfactant-rich phase, and the aqueous phase was easily decanted by simply inverting the tube. The micellar extract from this procedure was diluted with 0.2 ml of acetonitrile and transferred into a 0.5 ml quartz cell to record its absorbance at 538 nm.

Pretreatment of samples

Analysis of water (tap, Waste and river) samples for determination of copper ions content was performed as following: 400 ml of sample was poured in a beaker and 8.0 mL concentrated HNO_3 and 3.0

ml of 30% (v/v) H_2O_2 for elimination and decomposition of organic compound were added. The samples, while stirring was heated to one tenth volume. After adjustment of samples pH to desired value, the complexation and CPE were performed according to described general procedure.

Homogenized soil sample 20g or human blood from Health individuals sample (20 ml) was accurately taken and in a 200 ml beaker was digested in the presence of an oxidizing agent with addition of 10 ml concentrated HNO_3 and 2.0 ml 70% of $HClO_4$ was added and heated for 1.0 h. The content of beaker was filtered through a Whatman No. 40 filter paper into a 250 ml measuring flask and its pH was adjusted to desired value and diluted to mark with bi-distilled water. Then the above general procedure was applied. Copper levels in the final solutions were determined by ICP-AES and the above general procedures.

Spinach and lotus samples were purchased from Benha, Egypt. Afterwards, it was taken in small mesh. For the digestion of the sample, the procedure given by Ghaedi [43,44] was applied. A 40g of spinach and lotus samples were heated in silica crucible for 3.0 h on a hot plate and the charred material was transferred to furnace for overnight heating at 650 °C. The residue was cooled, treated with 10 ml concentrated nitric acid and 3.0 ml 30% H_2O_2 again kept in furnace for 2.0 h at the same temperature so that no organic compound traces are left. The final residue was treated with 3.0 ml concentrated hydrochloric acid and 2.0-4.0 ml 70% perchloric acid and evaporated to fumes, so that all the metals change to respective ions. The solid residue was dissolved in water, filtered and by keeping the pH at 4.5 made up to 25 ml by addition of thiel buffer. The dissolved solution was suitably diluted and presented procedure was applied.

A 50g of goat liver and/or meat were taken and dried for 48 h in an oven at 120 °C to remove the water content and to obtain a constant weight (about 68% water). Dried goat liver and/or meat sample were transferred into a glass flask. For the digestion of the sample, the procedure given by Ghaedi, et al. [43] was applied and the samples were treated according to previous publication [45,46]. A concentrated acid mixture of 3.0 ml H_2SO_4, 15 ml $HClO_4$, and 15 ml HNO_3 was added and left to stand over night. The solution was kept in an oil bath at 50 °C until the foaming stopped. Then the temperature was increased to 150 °C and heating was continued until the evolution of brown fumes of nitrogen oxides ceased. When a dark brown in mixture was appeared, the flask was cooled for about 2.0 min then a 5.0 mL of nitric acid had to be added. Heating was continued until nitrogen oxides fumes were longer given off. Appearance of white fume of perchloric acid in 1.0 ml solution is an indication of complete digestion. The solid residue was dissolved in water and filtered and by keeping the pH at 4.5 made up to 25 ml by addition of thiel buffer. Then the procedure was applied. Copper levels in the final solutions were determined by ICP-AES and the above general procedures.

Results and Discussion

Aluminon represents maximum absorbance at 336 nm at pH 3.5. Cu(II) in buffer medium of pH 3.5 reacts with aluminon in absence (λ_{max} 518 nm) and in the presence of Triton X-114, the absorbance of solution presented a bathochromic shift to 538 nm. Therefore the ternary complex of Cu^{2+}- aluminon - Triton X-114 can be extracted by CPE method. After separation of surfactant-rich phase, the absorbance was measured at 538 nm against a reagent blank as the reference (Figure 1). Therefore, the method is suitable for preconcentration and spectrophotometric determination of copper.

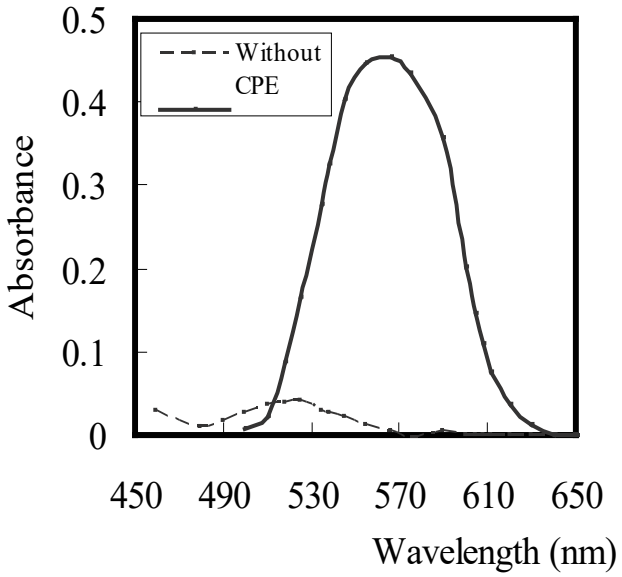

Figure 1: Absorption spectra for 150 ng/ml Cu^{2+} complexed with 2 × 10^{-5} M aluminon using 0.15% Triton X-114 at pH 3.5 with CPE and for 1.5 mg/ml without CPE.

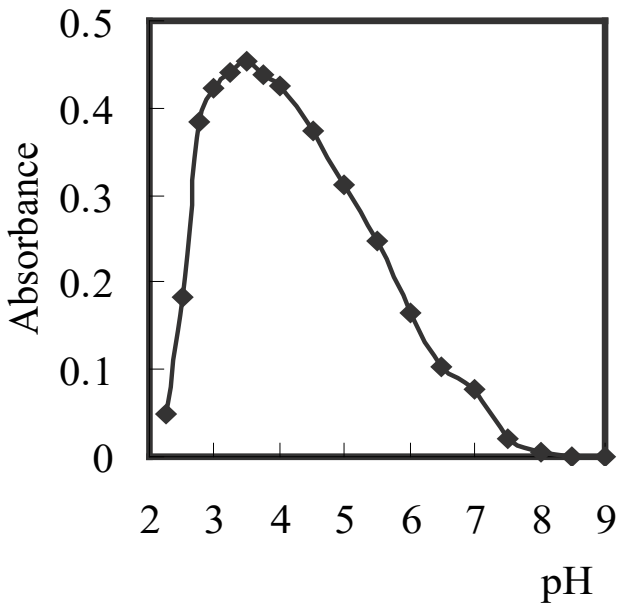

Figure 2: Effect of pH of on complexation of 150 ng/ml Cu^{2+} with 1.5×10^{-5} M aluminon using 0.15% Triton X-114 (SD = ± 0.06).

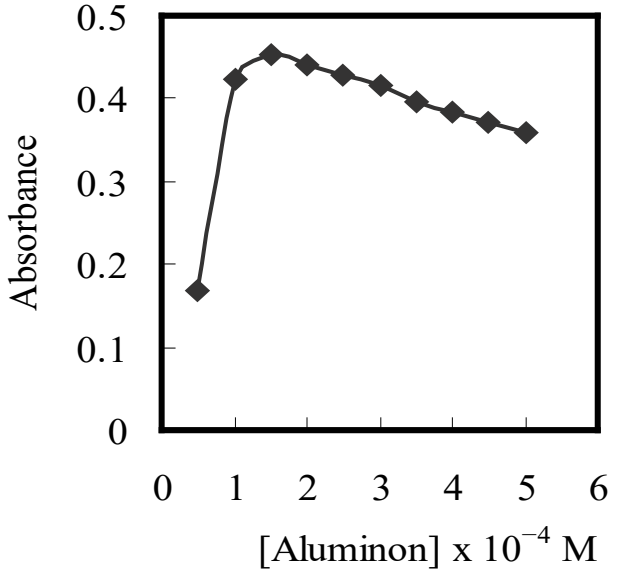

Figure 3: Effect of aluminon concentration on the complexation of 150 ng/ml Cu^{2+} using 0.15% Triton X-114 at pH 3.5 (SD = ± 0.04).

Optimization of the system

To take full advantage, the reagent concentrations and reaction conditions must be examined and optimized. Various experimental factors were illustrated in order to achieve optimization. These factors were optimized by setting all parameters to be constant and optimizing one each time.

The formation of Cu^{2+}-complex and its chemical stability are the two important influence parameters for CPE. The pH plays a unique role on Cu^{2+}-complex formation and subsequent extraction, and is proved to be a main factor for CPE. Extraction yield depends on the pH at which complex formation is carried out. The influence of pH on the absorbance at a constant concentration of complex in surfactant-rich phase was established in the range of 2.5-11.5. Various types of buffers (acetate, borate, phosphate, thiel, and universal) [42] were tested. The optimum one was the universal buffer, and the best values were between pH 3.0-4.0 (Figure 2). As Figure 2 shows, the pH 3.5 was selected for all further investigates, since the results is higher concordant at this value. The complexation reaction at pH values lower than 2.75 is incomplete due to protonation of reagent and complexation reaction is incomplete. The decrease in absorbance at pH values higher than 8.0 could be due to the hydrolysis of Cu^{2+}. Moreover the optimum volume of pH 3.5 per 100 ml sample was found to be 5.0 ml, since the results is highly concordance at this volume.

The influence of concentration of the complexing agent on the analytical responses was subsequently illustrated. The effect of aluminon concentration on the extraction and detection of Cu^{2+} was established in the range $(0.5\text{-}5.0) \times 10^{-4}$ M. The sensitivity of the procedure increased by increasing aluminon concentration up to 1.5×10^{-4} M and remained constant at higher concentrations. Therefore, 1.5×10^{-4} M of aluminon was applied in further works. The results are given in Figure 3.

It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature as a compromise between completion of extraction and efficient separation of phases. The dependence of extraction efficiency upon equilibration temperature and time above the cloud point in the range of 30-60 °C (Figure 4), and 5.0-25 min, were thoroughly optimized, respectively. The results represented that an equilibration temperature of 50 °C and an equilibration time of 5.0 min were adequate to achieve quantitative extraction. It was found that 50 °C is adequate for these analyses. An equilibration time of 5.0 min was selected as the optimal to reach maximum sensitivity. Unreasonably high temperatures are not suitable in the proposed analytical procedure since they could create stability problems for complexes and com-

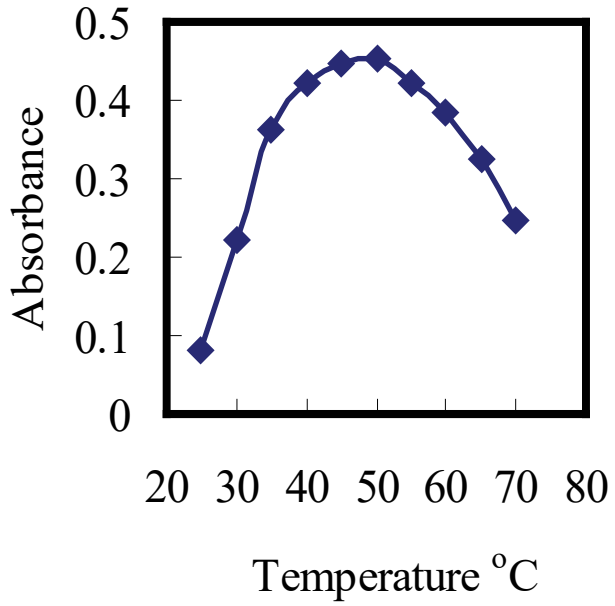

Figure 4: Temperature effect on the complexation of 150 ng/ml Cu(II) with 1.5 × 10^{-5} M aluminon using 0.15% Triton X-114.

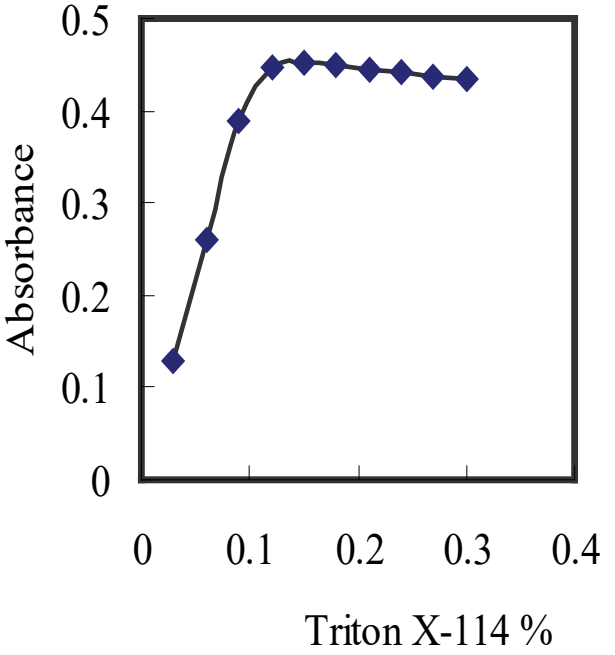

Figure 5: Effect of Triton X-114 concentration on the complexation of 150 ng/ml Cu^{2+} with 2.0 × 10^{-4} M aluminon at pH 3.5 (SD = ± 0.04).

plexing agents, while at higher temperature, CMC of non-ionic surfactants decreases [47]. The results indicate the experiment in the optimized reagent concentration after heating for 5.0 min at 50 °C and centrifuging by 5.0 min in 4000 rpm and cooling in 5.0 min in ice-bath lead to high recovery of Cu^{2+} ion in short time. Moreover, non-ionic surfactants appear relatively more hydrophobic at higher temperatures, due to an equilibrium shift that favors dehydration of the ether oxygen's [48]. This leads to an increase in the micelles concentration. The solubilization capability of the micellar solution increases with temperature leading to an increase in the complex extraction [48].

The concentration of surfactant that is used in CPE is critical parameter. To obtain the optimal concentration of Triton X-114, the influence of surfactant concentration on the increase in the absorbance of the system was established in the range 0.025-0.25% (v/v). As Figure 5 represents, the absorbance of the solutions increased by increasing the Triton X-114 concentration up to 0.15% (v/v) and remained constant at higher concentrations. The increase in absorbance by increasing surfactant concentration can be due to both more efficient extraction of the complex and medium effect. Therefore, 0.15% (v/v) Triton X-114 was used in further investigates.

As the surfactant-rich phase was very viscous, acetonitrile was added to the surfactant-rich phase after CPE to facilitate its transfer into spectrophotometric cell. The amount of 0.2 ml acetonitrile was chosen to have an appropriate amount of sample for transferring and recording the sample absorbance. Therefore, a preconcentration factor of 500 was obtained.

Stoichiometric ratio

The nature of the complex was illustrated at the optimum conditions described above using the molar ratio and continuous variation methods. The plot of absorbance versus the molar ratio of aluminon to Cu^{2+}, obtained by varying the aluminon concentration, showed inflection at molar ratio 2.0 (Figure 6), indicating presence of two aluminon molecules in the formed complex. Moreover, the Job method represented a ratio of aluminon to Cu^{2+} = 2.0. Consequently, the results showed that the stoichiometric ratio was (2:1) [aluminon:Cu^{2+}]. The conditional formation constant (log K), evaluated using Harvey and Manning equation applying the data obtained from the above two methods, was brought to be 4.37, whereas the true constant was 4.48.

For ternary complexes of Cu- aluminon -Triton X-114, the stoichiometric ratio as achieved from

molar ratio represented the formation of 1:1 for [Cu(aluminon)$_2$] : Triton X-114; so we conjectured that ternary complex [Cu(aluminon)$_2$] [Triton X-114] is formed in the system. The structure of which is probably as follows:

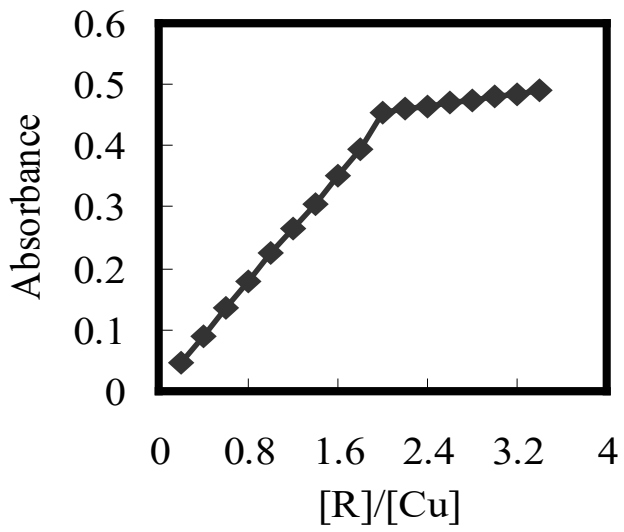

Figure 6: Molar ratio method for complexation of 2.0 × 10^{-4} M aluminon with Cu^{2+} at pH 3.5.

Cu^{2+} + 2 aluminon [Cu-(aluminon)$_2$]

[Cu-(aluminon)$_2$] + [Triton X-114] {[Cu-(aluminon)$_2$][Triton X-114]}

Calibration curve and sensitivity

The calibration curve showed that the system obeys Beer's law in the concentration range of 7.5-220 ng Cu^{2+} per ml in the measured solution (working range). For more accurate results, Ringbom optimum concentration ranges was obtained by plotting transmittance percentage against the logarithmic value of Cu^{2+} concentration and found to be 25-205 ng Cu^{2+}per ml in the measured solution. The linear regression equation obtained was A = 3.02 C (µg/ml) + 0.005 (r = 0.9996). The molar absorptivity was evaluated to be 1.92 × 10^7 L/mol.cm at 538 nm, whereas Sandell sensitivity was found as 0.0033 ng/cm^2. Because the amount of Cu^{2+} in 100 ml of sample solution is measured after preconcentration by CPE in a final volume of 0.2 ml, the maximum Enrichment factor of the solution is 500. The improvement factor, defined as the ratio of the slope of the calibration graph for the CPE

Table 1: Analytical features of the proposed method.

Parameters	After CPE	Before CPE
Amount of ethanol	0.2	---
pH	3.5	3.5
Optimum [aluminon]	1.5 × 10^{-4}	3.0 × 10^{-4}
Reaction time (min)	5.0	20
Stirring time (min)	5.0	----
λ_{max} (nm)	538	518
Working range (mg/ml)	7.5 - 220	5000 - 235000
Ringbom range (ng/ml)	25 - 205	7500 - 200000
Molar absorptivity (L/mol.cm)	1.92 × 10^7	1.65 × 10^4
Sandell sensitivity (ng/cm^2)	0.0033	0.45
Regression equation [a]		
Slope	3.02	0.0027
Intercept	0.005	- 0.009
Correlation coefficient (*r*)	0.99926	0.9965
RSD [a] (%)	1.75	3.90
Detection limits (ng/ml)	2.20	1500
Quantification limits (ng/ml)	7.40	4950
Preconcentration factor	500	---
Improvement factor	1165	---

[a]: A = a + bC, where C is the concentration of Cu^{2+} in µg/ml.

procedure to that of the calibration graph in aqueous media, for Cu^{2+} was 1165 (Table 1).

The standard deviations of the absorbance measurements were calculated from a series of 13 blank solution. The limits of detection (K = 3) and of quantification (K = 10) of the method were established [49] and recorded in Table 1, according to the IUPAC definitions ($C_1 = KS_o/s$ where C_1 is the limit of detection, S_o is the standard error of blank, s is the slope of the standard curve and K is the constant related to the confidence interval). The relative standard deviation was 1.85% obtained from a series of 10 standards each containing 125 ng/ml of Cu^{2+}.

The separation occurred efficiently, resulting in good enrichment factor and low LOD. In contrast to some other preconcentration techniques, no organic solvent is employed; therefore, the environmental pollution is limited to a small amount of surfactant. The methodology offers a facile, sensitive, inexpensive and nonpolluting alternative to other preconcentration techniques. The method is relatively rapid as compared with previously reported procedures for the enrichment of analyte ions (Table 2). The detection limits of analytes are superior to those of preconcentration techniques [50-56].

The sensitivity expressed as molar absorptivity of the proposed method is compared with those of published spectrophotometric methods (Table 3). The proposed method is more sensitive than other methods [57-70], that based on spectrophotometry (Table 3).

Interference

In order to study the selective separation and detection of copper ions from its binary mixtures with diverse metal ions, an aliquot of aqueous solutions (100 mL) containing 125 ng Cu^{2+} and mg amounts of other cations was taken and the proposed procedure was followed. The tolerance limit was taken as the ratio of foreign ions to the Cu^{2+} ion determined that causes a ± 5.0% error in the absorbance value. The results are summarized in Table 4. The result shows that most common ions do not interfere with the determination suggesting the highly selectivity of the proposed procedure.

Analytical applications

In order to confirm the applicability of the proposed procedure, it has been used to determine nanogram amounts of Cu^{2+} in environmental, in biological and water samples. The reliability of the presented procedure was checked by spiking experiments and independent analysis. The results

Table 2: Comparative data from some recent studies on preconcentration-separation of copper.

Technique	System	PF	Detection limit	Ref.
Slotted tube atom trap	-	-	30 µg/L	[50]
CPE	1-(2-Thenoyl)-3,3,3-trifluoraceton/ Triton X-114	96	0.4 µg/L	[51]
SPE	Amberlite XAD-4 functionalized with 3,4-dihydroxybenzoic acid	91	0.1 µg/L	[52]
Precipitation	2-[2`-(6-methylbenzothiazolylazo)] -4-aminophenol	40	0.5 µg/L	[53]
SPE	Silica-coated magnetic nanoparticles (SCMNPs) modified with γ- mercaptopropyltrimethoxysilane	476	0.092 µg/L	[54]
CPE	4-(Phenyl diazenyl)benzene-1,3- diamine/Triton X-114	30	0.6 µg/L	[55]
CPE	2-(2_-Benzothiazolylazo)-5-(*N*,*N* diethyl)aminophenol/Triton X-114	29	0.1 µg/g	[56]
CPE	triammonium salt of aurintricarbox- xylic acid		2.2 ng/L	This work

CPE: Cloud Point Extraction; SPE: Solid Phase Extraction; PF: Preconcentration Factor.

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Table 3: Comparison of selected reagents for the spectrophotometric determination of copper.

Reagent	λ_{max} (nm)	$\varepsilon \times 10^4$ L/mol.cm	Linear range (µg/ml)	Ref.
3,3`-(1,3-Propanediyldiimine)bis-[3-methyl-2-butanone] dioxime	525	2.95	0.5- 350	[57]
Bis(acetylacetone)ethylenediimine	370	--	up to 80	[58]
2-(5-Bromo-2-pyridylazo)-5-diethylamino amino phenol, polyglycol octylphenyl ether	560	15	0.0-18	[59]
1,5-Bis(di-2-pyridylmethylene) thiocarbonohydrazide	500	4.2	--	[60]
Diethyldithiocarbamate, β-cyclodextrin	436	1.3	0-6.0	[61]
3-{2-[2-(2-Hydroxyimino-1-methyl-propylideneamino)-ethylamino]-ethyl-imino}-butan-2-one oxime	570	0.16	0.2-225	[62]
Poly[allylamine-*co*-*N*-4-(8-aminoquinolyl -5-azo) benzylideneallylamine]	590	4.1	0-l.0	[63]
Dimethylindodicarbocyanine	650	12	0.0-1.2	[64]
1-[Pyridyl-(2)-azo]-naphthol-(2), TX-100, *N*,*N*`-diphenylbenzamidine up	520	11.4	to 0.6	[65]
S,*S*`-bis(2-aminophenyl)oxalate	504	0.54	0.4-150	[66]
Thiomichlersketone, polyethylene octyl phenyl ether	500	5.7	0-0.6	[67]
Naphthazarin	330	1.84	up to 4.5	[68]
bromosulphonazo III	616.8	33	0-1.024	[69]
1-Phenyl-1,2-propanedione-2-oxime thiosemi-carbazone	465	0.56	0.38-7.63	[70]
Triammonium salt of aurintricarboxylic acid	538	192	0.0075 - 0.22	This work

Table 4: Tolerance ratio of diverse ions on the determination of 150 ng/ml Cu^{2+} (relative error ± 5.0 %).

Ion added	Tolerance ratio ($w_{ion}/w_{Cu(II)}$)
K^+, Li^+, Na^+, succenate, acetate, tartaric acid	15000[a]
Ag^+, Ba^{2+}, Ca^{2+}, oxalic acid, $C_2O_4^{2-}$	12000
Ba^{2+}, Ca^{2+}, Mg^{2+}, S, Sr^{2+}, I^-, Br^-, B(III),	10000
Al^{3+}, Cr^{3+}, SO_4^{2-}, PO_4^{3-}, F^-	8750
Sn(IV), V(V), Cl^-, NO_3^-	7000
Co^{2+}, Cd^{2+}, borate, hydrazine,	5500
La^{3+}, Y^{3+}, Sc^{3+}, Br^-, hydrazine	4000
Fe^{2+}, Hg^{2+}, ClO_3^-	2500
Mn^{2+}, Zn^{2+}, SCN^-, SO_3^{2-}	1250
Mo(V), Sb(III), thiourea	750
Fe^{3+}, NO_2^-	400

[a]: Maximum limit tested.

for this study are presented in Table 5. An ICP-AES method was applied as a reference method and the results are also shown in Table 5. The recovery of spiked samples is satisfactorily reasonable and was confirmed using standard addition method, which indicates the capability of the system in the determination of ions. A good agreement was achieved between the added and measured analyte amounts. The recovery values evaluated for the added standards were always higher than 95%, thus confirming the accuracy of the method and its independence from the matrix effects.

The performance of the proposed method was assessed by calculation of the t-value (for accuracy) and F-test (for precision) compared with ICP-AES method. The mean values were obtained in a Student's t- and F-tests at 95% confidence limits for five degrees of freedom [71]. The results represented that the calculated values (Table 5) did not exceed the theoretical values. A wider range of determination, higher accuracy, more stability and less time consuming, represents the advantage of the proposed over other methods.

Table 5: Recovery studies of copper in real samples.

Sample	Added (ng/ml^{-1})	Found[a] (ng/ml)		RSD (%)	Recovery (%)	*t*- test[b]	*F*- value[b]
		Proposed	ICP-AES				
Spinach	0	28.9	28.8	-	-	-	-
	50	79.2	76.8	1.3	100.4	0.87	2.12
	100	127.5	131.5	1.6	98.9	1.11	2.60
Liver	0	29.1	29.0	-	-	-	-
	75	105.6	111.7	1.1	101.4	0.78	1.93
	150	177.4	185.3	1.4	99.1	1.19	2.92
Meat	0	30.6	30.5	-	-	-	-
	60	89.9	88.8	1.7	99.2	0.96	2.13
	120	212.0	154.7	1.5	101.0	1.10	2.54
Lotus (tree)	0	17.9	17.8	-	-	-	-
	90	107.1	110.6	1.1	99.3	1.23	3.18
	180	200.2	192.3	1.5	101.2	1.15	2.78
Soil	0	18.3	18.1	-	-	-	-
	80	99.5	95.6	1.4	101.2	1.24	3.19
	160	177.3	180.9	1.2	99.4	0.90	2.02
Blood	0	77.5	77.5	-	-	-	-
	40	120.4	120.0	1.6	102.5	1.17	2.85
	80	155.2	153.7	1.8	98.5	1.64	3.47
Tap water	0	6.5	6.3	-	-	-	-
	100	106.3	106.5	1.7	99.8	0.82	1.99
	200	208.6	203.1	1.5	101.0	1.09	2.47
Waste water	0	75.6	75.5	-	-	-	-
	70	148.7	142.3	1.2	102.1	0.98	2.20
	140	213.5	218.7	1.4	99.0	1.24	3.11
River water	0.0	63.2	63.0	-	-	-	-
	65	130.4	125.0	1.6	101.7	1.45	3.32
	130	196.5	197.8	1.4	101.7	1.37	3.23

[a]: Average of six determination; [b]: Theoretical values for *t* and *F* at 95% confidence limit are 2.57 and 5.05, respectively.

Conclusions

Cloud point extraction was applied to the preconcentration of copper in various samples. The procedure, which is based on the cloud point extraction of the complex of copper with aluminon, allows the determination of copper as low as 2.2 ng/ml. The proposed procedure needs inexpensive instrumentation and offers safety, good selectivity, accuracy and precision that can be used to the determination of copper in real samples. The surfactant has been applied for preconcentration of copper in samples, and thus toxic solvent extraction, has been avoided. The methodology offers a simple, rapid, inexpensive and nonpolluting alternative to other preconcentration techniques. The procedure is relatively rapid as compared with previously reported procedures for the enrichment of analyte ions.

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