Selective Flotation-Spectrophotometric Determination of Trace Copper(II) in Natural Waters, Human Blood and Drug Samples Using Phenanthraquinone Monophenylthiosemicarbazone

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Received July 19, 2000; accepted February 19, 2001

Copper(II) forms 1 : 1 and 1 : 2 intense red complexes with phenanthraquinone monophenylthiosemicarbazone (PPT) at pH 3—3.5 and ≥6.5, respectively. These complexes exhibit maximal absorbance at 545 and 517 nm, the molar absorptivity being 2.3×10^4 and 4.8×10^4 l mol^{-1} cm^{-1}, respectively. However, the 1 : 1 complex was quantitatively floated with oleic acid (HOL) surfactant in the pH range 4.5—5.5, providing a highly selective and sensitive procedure for the spectrophotometric determination of Cu^{II}. The molar absorptivity of the floated Cu–PPT complex was 1.5×10^5 l mol^{-1} cm^{-1}. Beer's law was obeyed over the range 3—400 ppb at 545 nm. The analytical parameters affecting the flotation process and hence the determination of copper traces were reported. Also, the structure of the isolated solid complex and the mechanism of flotation were suggested. Moreover, the procedure was successfully applied to the analysis of Cu^{II} in natural waters, serum blood and some drug samples.

Key words copper; phenanthraquinone monophenylthiosemicarbazon; flotation; spectrophotometry; blood, complex and drug

Copper is an essential constituent of about thirty enzymes and glycoproteins and is required for the synthesis of haemoglobin.[1—3] It promotes iron absorption from the gastrointestinal system, is involved in the transport of iron from tissues into plasma, helps to maintain myelin in the nervous system, is important in the formation of bone and brain tissues and is necessary for other important functions.[2] When levels of copper exceed certain values, however, the defense mechanisms protecting the body against its excess are overcome and toxicity results.

The list of toxic copper species[4,5] often includes $CuOH^+$, $CuCO_3$ and $Cu_2(OH)_2^{2+}$. However, without question, Cu^{2+} (which is present in various aqueous systems) is known to be toxic and its toxicity is pH dependent.[5—7] Around us, copper is present in seawater in a trace concentration[8] that ranges between 0.05 and 0.1 mg dm^{-3}; in potable water the concentration is <0.05 mg dm^{-3} while the upper limit[9] reaches 1.5 mg dm^{-3}. Accordingly, from the viewpoints of pollution, environmental chemistry, geochemistry, marine biology and analytical control in industrial, food, agricultural, pharmaceutical and clinical areas, it is necessary to establish a rapid, simple, sensitive and accurate procedure for the preconcentration of Cu^{II} prior to its determination. In this concern, numerous techniques for the separation and concentration of trace metals including evaporation of solvents, electrodeposition, liquid–liquid extraction, surface adsorption, precipitation, ion exchange, ion exchange impregnated materials, immobilized reagents, electro-osmosis and flotation have been reported.[10—12] Although some of these techniques may be tedious, having limited concentration factors, lengthy and rigid conditions for the preparation of solid adsorbents,[10] the flotation technique (which is the choice for this investigation) has many advantages that overcome these drawbacks.

Spectrophotometry still represents an attractive technique for the determination of metal ions in aqueous media because of its simplicity, being inexpensive and more readily available. Although a vast number of reagents are available for the spectrophotometric determination of copper,[8,13—15] little work has been done using PPT[16] and perhaps no trial has been made to float the analyte with this reagent. The present investigation aims to develop a simple, rapid and selective procedure for the preconcentration and determination of Cu^{II} in natural waters, blood and drug samples using HOL as a surfactant and PPT as a chelating agent. The procedure involves the spectrophotometric determination of the analyte directly in the scum, thus eliminating matrix interference and increasing the sensitivity.

Experimental

Unless otherwise specified, all chemicals used were of analytical-reagent grade. Doubly distilled water was used throughout. Phenanthraquinone monophenylthiosemicarbazone (PPT), Chart 1, was synthesized, as described elsewhere,[16] by refluxing equimolar amounts of ethanolic 4-phenyl-3-thiosemicarbazide with 9,10-phenanthraquinone dissolved in the least amount of glacial acetic acid. The purity of the reagent was checked by C, N and H analysis and infrared spectrometry. The product is crystalline (mp 198 °C), sparingly soluble in ethanol and/or methanol but easily soluble in acetone, DMF and DMSO; 1×10^{-4} mol dm^{-3} ethanolic solution of PPT was prepared and used in all measurements. Oleic acid (HOL), being an inexpensive substance that forms an organic layer which facilitates spectrophotometric measurements, has been chosen as a surfactant. HOL stock solution, 6.36×10^{-2} mol dm^{-3}, was prepared by dispersing 20 cm^3 of HOL (food grade with sp. gr. 0.895, provided by JT Baker Chemical Co.) in 1 dm^3 kerosene. Copper(II) stock solution was prepared by dissolving the requisite amount of $CuCl_2\cdot2H_2O$ in doubly distilled water and its exact concentration was determined by AAS.

Sample Collection and Pretreatment One cubic decimeter of tap, Nile or seawater samples was filtered off, adjusting the pH to 1 with concentrated HCl, to prevent losses by sorption or coprecipitation, and preserved in high quality clean plastic containers.

Centrum, a multimineral general tonic containing copper, was supplied by MUP which manufactures the drug under the license of American Cyanamid Company's Lederle Laboratories. Gerimax® is produced under pharmaceuti-

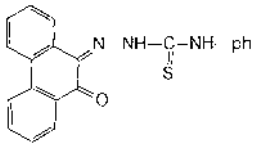

Chart 1

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cal control by Danks Droge ALS for B. light, Denmark. Exactly 0.02 g of each drug sample was taken, crushed and heated in 5 cm^3 concentrated HNO_3 for dissolution. The cold solution was filtered, collected in a 50-cm^3 calibrated flask and filled to the indicated mark with doubly distilled water.

Blood samples (5 cm^3, each) were collected from 30 healthy occupationally non-exposed adult males (Mansoura City, Egypt). Each sample was centrifuged and the supernatant fluid (serum) was aspirated. Each serum sample was acidified using 2 cm^3 of $HClO_4 : HNO_3$ (1 : 3) mixture, boiled to near dryness, dissolved in doubly distilled water and brought up to 5 cm^3 in a calibrated flask.

Exactly 0.2 g of each Cu^{II} complex sample (kindly supplied by the laboratory of Pro. Dr. M. E. Bekheit, Professor of Inorganic Chemistry, Mansoura University), was dissolved in 5 cm^3 HNO_3, heated until evaporation, cooled and filled to 50 cm^3 in a calibrated flask with doubly distilled water.

Apparatus Flotation Cells: Two types of flotation cells were used throughout this work, as has already been described.[14] Flotation cell (a), is a cylindrically graduated glass tube of 16 mm inner diameter and 290 mm length with a stopcock at the bottom. Such a cell is used to study the different factors affecting the efficiency of flotation. Flotation cell (b) is a cylindrical tube of 6 cm inner diameter and 45 cm length with a stopcock at the bottom and a quick fit stopper at the top; this cell is used to separate copper from 1 dm^3 of different water samples. The concentration of Cu^{II} was determined using a Unicam UV 2100 UV/vis spectrophotometer, a Griffin Model 40 colorimeter and was confirmed by AAS measurements at 324.7 nm with a Perkin–Elmer 2380 atomic absorption spectrometer. The infrared data were recorded on a MATTSON 5000 FTIR spectrophotometer. The pH of the solutions was measured using a Hanna Instruments 8519 digital pH meter.

General Procedure Separation: Two cubic centimeters of 1×10^{-4} mol dm^{-3} PPT in ethanol was introduced into a flotation cell containing 2×10^{-6} mol dm^{-3} Cu^{II}, then the pH was adjusted to around 5 using HCl and/or NaOH and the solution was mixed thoroughly. The mixture was then diluted to 10 cm^3 with an alcohol–water mixture to ensure a final ethanol volume fraction of 30%. To the above solution 3 cm^3 of oleic acid with a definite concentration (specified for each experiment) was added. The cell was then turned upside down twenty times by hand and kept upright for 5 min to ensure complete flotation of the colored Cu–PPT species.

Determination: A suitable volume of the floated layer was separated and introduced into a quartz cell for spectrophotometric or colorimetric determination of Cu^{II} at 545 nm using 1×10^{-4} mol dm^{-3} PPT as a reagent blank. The analyte concentration was calculated from a calibration curve constructed by taking different concentrations of Cu^{II} covering the basic range up to 0.3 ppm copper. The data obtained were confirmed by AAS after trapping Cu–PPT complex in the aqueous phase, to avoid the hazardous effect of HOL on the AAS signal. Eluting the HOL layer with 5 cm^3 of HNO_3 (1 : 1) solution completed trapping. The separation efficiency (% F) was calculated from the relation:

$$\% F = (C_s/C_i)\times 100\ \%$$

here, C_s and C_i denote the scum and the initial concentrations of Cu^{II}, respectively.

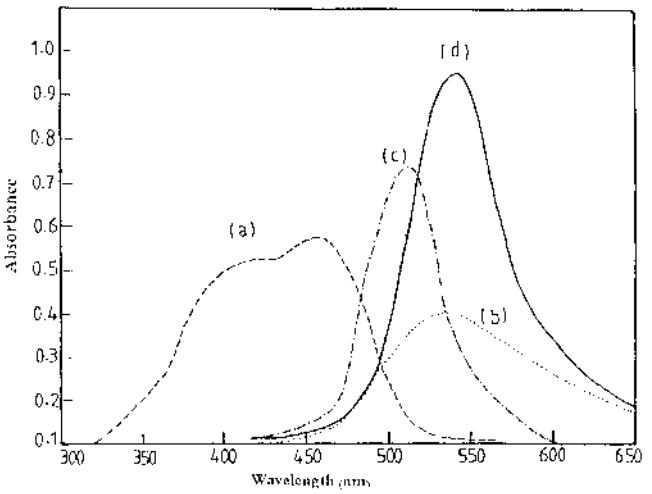

Fig. 1. Absorption Spectra

(a) The reagent PPT at pH 5.0; (b) Cu–PPT mixture at pH 3.0; (c) Cu–PPT mixture at pH 6.5 and (d) Cu–PPT–HOL system at pH 5.0.

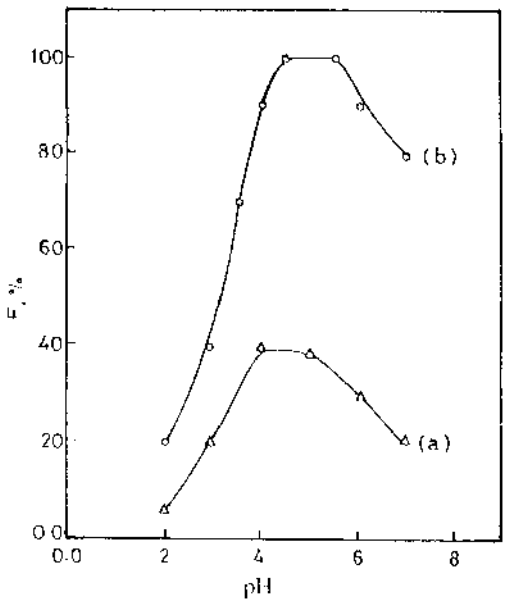

Fig. 2. Floatability of Cu^{II} *vs.* pH

Cu^{II}, 2×10^{-6} mol dm^{-3}; PPT, 2×10^{-5} mol dm^{-3} and HOL, 2×10^{-4} mol dm^{-3} HOL in the absence (curve (a)) and presence (curve (b)) of PPT.

Results and Discussion

Work conditions were set up after many trials. The preliminary investigations indicated that the reaction of PPT with Cu^{II} is pH dependent. At pH 3.0 copper(II) reacts with PPT forming an intense red complex which absorbs at 545 nm, while at pH $\geq$6.5, the maximum absorbance is found to be at 517 nm. Moreover, the system Cu–PPT is quantitatively floated in the pH range 4.5—5.5 and the magnitude of absorbance at λ_{max} is taken as an indication of the efficiency of flotation.

Absorption Spectra The absorption spectra of the reagent PPT and the Cu–PPT complexes formed in aqueous solution and which floated into the HOL layer are shown in Fig. 1. As can be noticed, the absorption spectrum of the reagent PPT (1×10^{-4} mol dm^{-3}) in ethanol water mixture (30% v/v) is characterized by two maxima at 410 and 450 nm (curve (a)) which are attributable to the presence of keto-enol or thioenol equilibria.[16] Curves (b) and (c) represent the absorption spectra of Cu–PPT complex at pH 3 and 6.5, respectively. Also, the absorption spectrum of the Cu–PPT–HOL system after flotation with HOL surfactant at pH 5 is given (curve (d)). It is evident that the absorbance depends on the pH of solution and this can be ascribed to the formation of different Cu–PPT complex species. Moreover, the floated complex species has a higher absorbance value compared to that obtained in aqueous solution, a finding that enhances remarkably the sensitivity of spectrophotometric determination of Cu^{II} after flotation. Subsequent measurements were performed at 545 nm and pH 5.

Effect of pH The separation of 2×10^{-6} mol dm^{-3} Cu^{II} from aqueous solution as a function of pH was investigated using 2×10^{-5} mol dm^{-3} PPT and 2×10^{-4} mol dm^{-3} HOL. The results are shown in Fig. 2. Close inspection of the figure shows that the maximum flotation efficiency (*ca.* 100%) is attained in the pH range 4.5—5.5 (curve (b)). The role of

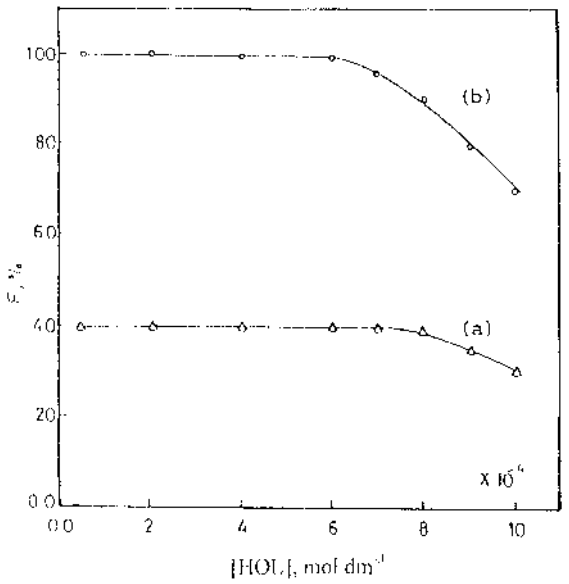

Fig. 3. Floatability of Cu^{II} *vs.* HOL Concentration

Cu^{II}, 2×10^{-6} mol dm^{-3}; PPT, 2×10^{-5} mol dm^{-3} at pH 5.0 in the absence (curve (a)) and presence (curve (b)) of PPT.

PPT in the separation (and hence determination) is evident from a comparison with curve (a) which represents the flotation of copper in the absence of PPT. Under these conditions the % F does not exceed 40%.

Effect of HOL Concentration The floatability of 2×10^{-6} mol dm^{-3} Cu^{II} using different concentrations of HOL (Fig. 3) in the absence (curve (a)) and in the presence (curve (b)) of 2×10^{-5} mol dm^{-3} PPT at pH 5.0 has been thoroughly investigated. The data obtained showed that the maximum flotation (*ca*. 100%) is obtained in a wide HOL concentration that ranges from 5×10^{-5} to 6×10^{-4} mol dm^{-3}. The role of PPT in the separation process is quite apparent from comparing curves (a) and (b). For subsequent measurements, 3 cm^3 of 2×10^{-4} mol dm^{-3} HOL was used.

Effect of PPT and Cu^{II} Concentrations The effect of PPT concentration in the range ($0.1—5\times10^{-4}$ mol dm^{-3}) on the flotability of 4×10^{-5} mol dm^{-3} Cu^{II} using 2×10^{-4} mol dm^{-3} HOL at pH 5.0 was investigated. Data obtained indicated that complete flotation of Cu^{II} is attained only if [PPT] is $\geq$[Cu], so during the flotation process excess amounts of PPT were added. Such excess in reagent concentration facilitates the application of the procedure to real samples. Further, another series of experiments was conducted to float different concentrations ($0.1—5\times10^{-5}$ mol dm^{-3}) of Cu^{II} using 2×10^{-5} mol dm^{-3} PPT in the presence of 2×10^{-4} mol dm^{-3} HOL at pH 5.0. The data obtained confirmed those found in the preceding experiments, *i.e*., *ca.* 100% flotation is obtained when the Cu: PPT ratio is $\leq$1. In most experiments in this investigation the concentration of PPT was chosen to be 10-fold that of Cu^{II}.

Effect of Temperature A series of experiments was conducted over a wide temperature range (20—80 °C) to determine the proper temperature required for maximum flotation of Cu–PPT complex. It was found that the flotation efficiency was not markedly affected in the 20—60 °C range. Therefore, subsequent measurements were carried out at room temperature, *i.e*., *ca*. 25 °C.

Effect of Foreign Ions In spite of the high tendency of PPT to form complexes with a great number of metal ions, fortunately most of those complexes are not formed at pH $\leq$6 (the recommended pH for this investigation was 5). This makes the determination of Cu^{II} in the presence of a great number of metal ions feasible without interference. Under the optimum conditions, 100-fold of Ca(II), Mg(II), Sr(II), Fe(III), Cr(III), Al(III), Cd(II), Zn(II), La(III), Pt(VI), Au(III), Hg(II), Ni(II), Co(II) and Mn(II) do not interfere. Only Pd(II) forms a colored chelate, which is not completely floated with Cu–PPT complex. Ten-fold of Pd(II) could be tolerated in the flotation and determination of 0.1 mg dm^{-3} of Cu^{II}.

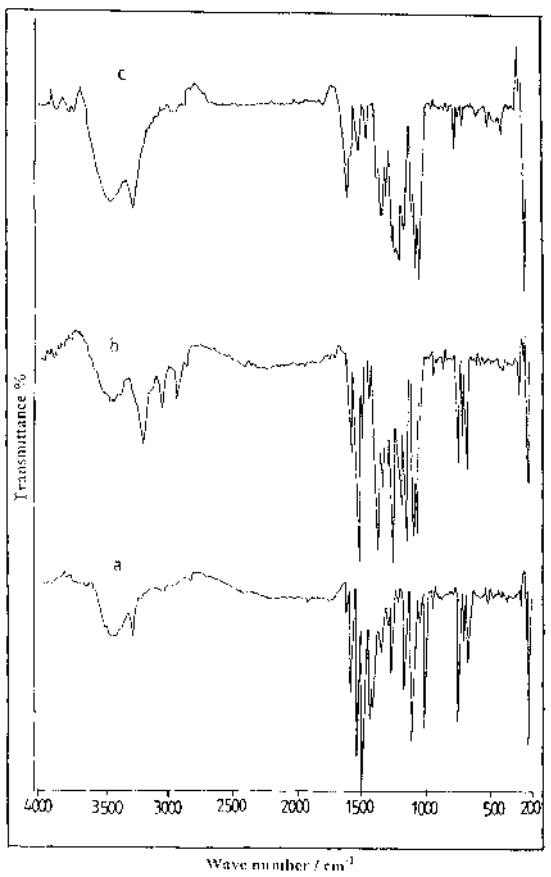

Fig. 4. Infrared Spectra

(a) The reagent PPT; (b) Cu–PPT complex and (c) Cu–PPT–HOL floated complex. The measurements were carried out in KBr discs.

Characterization of the Solid Complex Elemental analysis of the isolated solid complex indicates the formation of [Cu(PPT–H)Cl·2H_2O], C=51.2 (51.3), H=3.8 (3.7), Cu=13.2 (12.9), Cl=7.5 (7.2). The molar conductivity measurement in DMSO at 25 °C indicates the non electrolytic nature of the complex.

The infrared spectrum of PPT is characterized by the presence of bands at 1680 and 1630 cm^{-1} which were assigned to ν(C=O) and ν(C=N), at 790 and 1240 cm^{-1} assigned to ν(C=S) and the combination of ν(C=S) with ν(C=N),[17] at 3290 and 3080 cm^{-1} assigned to ν(N–H) stretching vibrations and at 3450—3500 cm^{-1} assigned to OH group, indicating the enolization of C=O group in the phenanthraquinone moiety to C–OH group.

Comparison of the IR spectrum of the Cu–PPT complex with that of PPT (Fig. 4) shows that PPT acts as a tridentate

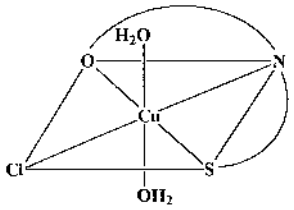

Chart 2

ligand in the enol thion form coordinating *via* the (C=S), (N=N) and the enolic carbonyl OH with the displacement of the hydrogen atom from the latter group. This mode of chelation is supported by: i) the disappearance of ν(C=O) and ν(C=N) bands assigned at 1680 and 1630 cm^{-1} in the spectrum of PPT; ii) the appearance of new bands at 1540—1545 and 1095 cm^{-1} assignable to ν(N=N)[18] and ν(C–O), respectively[19] and iii) the ν(C=S) bands shift to lower wave number and the appearance of new bands at 490, 420, 320, 290 cm^{-1} assignable to ν(Cu–O), ν(Cu–N), ν(Cu–S) and ν(Cu–Cl), respectively.[20] The presence of water in the coordination sphere is supported by the existence of bands at 3400, 1630, 900 and 630 cm^{-1} which are due to νOH $\delta(OH_2)$, $\rho_\nu(H_2O)$ and $\rho_w(H_2O)$ vibrations respectively.[21] All these observations suggest the following structure in Chart 2 for the [Cu(PPT–H)Cl·2H_2O]. Moreover, the electronic spectra of this complex exhibit a band centered at 18600 cm^{-1} (542 nm) which is probably due to charge transfer transition. This band matches well with the visible spectrum of Cu–PPT mixtures, indicating the identity of the isolated solid complex with that present in solution.

Flotation Mechanism First of all, it must be taken into consideration that oleic acid (HOL) begins to dissociate at pH ≥5.2.[22] Since the recommended pH for this investigation was ≤5, the HOL molecules share in flotation in the undissociated form. Accordingly, the flotation mechanism is proposed to proceed through hydrogen bonding between HOL and Cu–PPT system. This confirmation stems from the following experimental data and observations:

1) The floated species (Cu–PPT–HOL) have the same λ_{max} as those formed in aqueous solution (Cu–PPT).

2) Comparison of the infrared spectrum (Fig. 4) of the Cu–PPT complex isolated from the aqueous layer with that of the complex formed in oleic acid layer shows that all the bands are similar except those appearing at *ca*. 1820, 2050 and 2400 cm^{-1} in the spectrum of the latter. These bands are due to ν(O–H···O) vibrations of the intermolecular hydrogen bonding. Therefore, the system Cu–PPT–HOL became hydrophobic and floated with air bubbles to the surface of the solution. The schematic diagram of the flotation process is represented in Chart 3.

Composition of the Complex Formed in Solution Job's method of continuous variation[23] was applied to identify the stoichiometry of Cu–PPT complexes formed at pHs 3.5 and 6.5 in aqueous solutions. The data obtained indicate the formation of 1 : 1 (Cu : PPT) at pH 4.0 with λ_{max} at 545 nm and 1 : 2 at pH 6.5 with λ_{max} at 517 nm. The data were used to compute the stability constants which were found to be $1:23\times10^6$ ($\log K=6.09$) and 8×10^{11} ($\log K=11.93$), respectively. Most of the floated species at pH 5.0 have the stoichiometric ratio 1 : 1 (Cu–PPT).

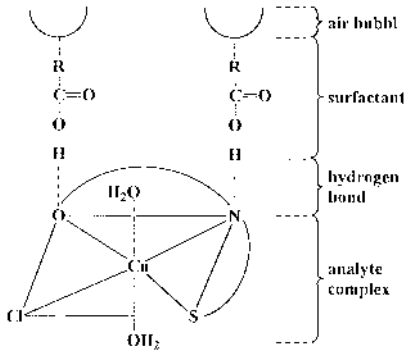

Chart 3

Table 1. Trace Analysis of Cu^{II} in Natural Water Samples by Spectrophotometric and AAS Techniques after Preconcentration Using 5×10^{-5} mol dm^{-3} PPT and 5×10^{-4} mol dm^{-3} HOL at pH 5.0 (n=5)

Sample (Location)	Cu^{II} (μg dm^{-3})	
	Spectrophotometric	AAS
Tap water (Mansoura city)	1.1±0.05 [3.3—5]	1.09±0.02
Nile water (Damiatta)	6.5±0.10	6.54±0.07
Lake water (Manzala)	3.8±0.08 [0.1—5]	3.86±0.03
Seawater (Ras Elbar)	5.2±0.06 [0.2—5.0]	5.25±0.02

The values between square brackets are average international values or values from previous work.[24]

Validity of Beer's Law and Reproducibility Under the optimum conditions, a linear calibration graph was obtained for 3.0—400 ng cm^{-3} Cu^{II} at 545 nm (r=0.993, n=5) with a molar absorptivity of 1.5×10^5 l mol^{-1} cm^{-1}. Such a sensitivity is very high compared with that obtained in the aqueous solution ($\varepsilon=2.3\times10^4$ l mol^{-1} cm^{-1}). The regression equation of the calibration graph obtained by the least squares method is $A=\log(P_o/P_t)=150287\,[Cu^{II}]+0.009$. The detection limit calculated from (2S+blank) value is found to be 50 ng cm^{-3} Cu^{II}.

Application

Water Samples The proposed procedure was successfully applied for the determination of Cu^{II} in tap, Nile, lake and seawaters. Into flotation cell (b) containing 0.75 dm^3 of water sample, 5 cm^3 of 1×10^{-4} mol dm^{-3} PPT is added and the pH is adjusted to 5.0. To this solution 5 cm^3 of 2×10^{-4} mol dm^{-3} HOL was added and the total volume was completed to 1 dm^3 with a water sample. Then, the flotation cell is turned upside down 20 times by hand to ensure complete flotation. The scum, 5 cm^3, is separated and measured spectrophotometrically at 545 nm for Cu^{II} determination. It is worth noting that a parallel series of the same experiment was carried out under the same conditions and that the copper content was completely eluted and introduced directly into the flame for AAS determination as a confirmatory test. The results of spectrophotometric and AAS determination are listed in Table 1. The experimental means of the two investigated methods using the null hypothesis of $|t|_2$ for p=0.05 and n=10 were carried out. Close inspection of the

Table 2. Analysis of Cu^{II} in Some Drugs Using 2×10^{-5} mol dm^{-3} PPT and 2×10^{-4} mol dm^{-3}HOL at pH 5.0 ($n=5$)

Drug	Mineral composition (mg/tablet)	Cu^{II} (mg/tablet)		
		Spectrophotometric	AAS	Recovery (%)
Gerimax	Mg (150), Fe (14), Zn (15), Mn (2.5), Cr (0.05), Se (0.05), Mo (0.15)	1.97±0.05 (2.0)	1.98±0.07	98.5
Centrum	Ca (162), F e(27), Mg (100), Mn (7.5), K (7.5), Zn (22.5)	2.93±0.06 (3.0)	2.93±0.10	98.3

Values in parentheses are calculated values.

Table 3. Determination of Cu^{II} in Some Representative Blood Serum Samples by Spectrophotometric and AAS Techniques Using 2×10^{-5} mol dm^{-3} PPT and 2×10^{-4} mol dm^{-3} HOL at pH 5.0 ($n=5$)

Sample	Cu^{II} (mg cm^{-3})	
	Spectrophotometric	AAS[a]
1	1.05±0.080	1.06±0.040
2	1.08±0.100	1.08±0.060
3	1.10±0.050	1.09±0.070
4	1.06±0.063	1.06±0.055
5	1.15±0.035	1.15±0.085
6	1.14±0.070	1.15±0.090

a) Considered as reference for statistical calculation.

Table 4. Determination of Copper in Some Complexes in the Presence of 2×10^{-5} mol dm^{-3} PPT and 2×10^{-4} mol dm^{-3} at pH 5.0 ($n=5$)

Compound[a]	Cu^{II} (μg)		
	Spectrophotometric	AAS	Recovery (%)
[Cu(HPAPTS)(OAc)(H_2O)]	14.2±0.03 [14.3][b]	14.2±0.05	99.3
[Cu(HPAPTS)Cl(H_2O)]	16.0±0.07 [15.8][b]	16.1±0.10	100.01
[Cu(HpxAPTS)(OAc)]	15.0±0.08 [15.2][b]	15.2±0.09	98.7
[Cu(H_2OAPT)Cl]	14.4±0.06 [14.0][b]	14.2±0.10	102.8

a) HPAPTS denotes 1-phenylacetyl-4-phenyl-3-thiosemicarbazide; HPxAPTS, 1-phenoxacetyl-4-phenyl-thiosemicarbazide; H_2OAPT; α-oximinoacetoacetyl pyridine-4-phenylthiosemicarbazone. *b*) Calculated values.

data shown revealed that the experimental data for the investigated analyte were in reasonable agreement with the comparable reference values.[24)]

Drug Samples The proposed procedure was applied to the recovery and determination of Cu^{II} in two types of drugs (*viz*. Centrum and Gerimax) under the recommended conditions. The results are listed in Table 2 and good recoveries were obtained.

Blood Serum Samples To apply the proposed procedure for the determination of Cu^{II} in human blood serum, 2 cm^3 of each serum sample was treated by the previously described procedure. The mean value of Cu^{II} in the human serum for the studied samples was found to be 1.1 ± 0.04 μg cm^{-3}, Table 3.

Determination of Cu^{II} in Some of Its Complexes The proposed procedure was successfully applied to the analysis of Cu^{II} in some of its complex samples. The results illustrated in Table 4 indicate good recovery through the determination of Cu^{II}.

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