

Extraction of trace copper(II) using C₁₈ membrane disks modified Graphene oxide with covalently linked porphyrin in water samples and biologics sample by FAAS

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Abstract

A simple, highly sensitive, accurate and selective method for determination of trace amounts of Cu²⁺ in water samples and paraffin-embedded tissues from Liver loggerhead turtles specimens is presented. A novel Graphene oxide with covalently linked porphyrin solid-phase extraction adsorbent was synthesized by covalently linked porphyrin onto the surfaces of graphite oxides. The rapid extraction and determination of trace amounts of copper(II) ions using octadecyl-bonded silica membrane disks modified by Graphene oxide with covalently linked porphyrin (GO-H₂P) and Atomic Absorption Spectrometry is presented. The method is based on complex formation on the surface of the ENVI-18 DISK™ disks followed by stripping of the retained species by minimum amounts of appropriate organic solvents. The elution is efficient and quantitative. The effect of potential interfering ions, pH, ligand amount, stripping solvent, and sample flow rate were also investigated. Under the optimal experimental conditions, the break-through volume was found to be about 1000 mL providing a preconcentration factor of 500. In the present study, we report the application of preconcentration techniques still continues increasingly for trace metal determinations by flame atomic absorption spectrometry (FAAS) for quantification of Cu in Formalin-fixed paraffin-embedded (FFPE) tissues from Liver loggerhead turtles. The maximum capacity of the disks was found to be 389 ± 4 µg for Cu²⁺. The limit of detection of the proposed method is 3 ng per 100 mL. The method was applied to the extraction and recovery of copper in different water samples.

Key words: Copper(II) ;SPE;C₁₈ disks; FAAS ; Graphene oxide with covalently linked porphyrin (GO-H₂P); Formalin-fixed paraffin-embedded (FFPE)

Full length article Received: 11-07-2013 Revised: 24-09-2013
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Accepted: 11-01-2014

Available online: 31-01-2014

1. Introduction

Cu at trace concentrations acts as both a micronutrient and a toxicant in marine and fresh water systems [1-5]. This element is needed by plants at only very low levels and is toxic at higher levels. At these levels, Cu can bind to the cell membrane and hinder the transport process through the cell wall. Cu at nearly 40 ng mL⁻¹ is required for normal metabolism of many living organisms [6]. On the other hand, Cu is an important element in many industries. Thus, the development of new methods for selective separation, concentration and determination of it in sub-micro levels in different industrial, medicinal and environmental samples is of continuing interest. The determination of Cu is usually carried out by flame and graphite furnace atomic absorption spectrometry (AAS) [7], as well as spectrometric methods [10]. Solid phase extraction (SPE) methods are the best alternatives for traditional classic methods due to selective removal of trace amounts of metal ions from their matrices. Solid phase extraction determinations can be carried out on different

efficient ways. One of the most appropriate performance features of SPE is achieved by using octadecyl silica membrane disks. SPE reduce the use of toxic solvent, disposal costs, and extraction time [8-11]. The octadecyl silica membrane disks involves shorter sample processing time and decreased plugging due to the large cross-sectional area of the disk and small pressure drop which allows higher flow-rates; reduced channeling resulting from the use of sorbent with smaller particle size and a greater mechanical stability of the sorbent bed [13]. In our previous attempts, we modified SPE membrane disks with suitable compounds for selective determination of chromium [14] and lead [11]. Meanwhile, other investigators have successfully utilized these sorbents for quantitative extraction and monitoring trace amounts of lead [15], copper [16], silver [19], mercury [17,19], cadmium [20], palladium [21], Ce [22] and UO₂ [12]. The structure of Graphene oxide with covalently linked porphyrin (GO-H₂P) is shown in Fig. 1. The chelated ions were desorbed and determined by FAAS. The modified solid phase could be used at least 50 times

with acceptable reproducibility without any change in the composition of the sorbent, GO-H₂P. On the other hand, in terms of economy it is much cheaper than those in the market, like C₁₈ SPE mini-column..

The optimized method was applied to Cu²⁺ determinations in different natural waters. The second aim of this study was the selection of an appropriate method for the analysis of FFPE tissue were based On present work with atomic absorption spectrophotometric determination of Cu.

2. Material and Methods

Reagents

All acids were of the highest purity available from Merck and were used as received. Methanol and Chlorofom were of HPLC grade from Merck. Analytical grade nitrate salts of lithium, sodium, potassium, magnesium, calcium, strontium, barium, zinc, cadmium, lead, nickel, cobalt(II), and copper(II) were of the highest purity. Ultra-pure organic solvents were obtained from E.Merck, Darmstat, Germany, and High Purity double distilled deionized water was used throughout the experiments. The stock standard solution of Cu²⁺ was prepared by dissolving 0.1000g of the copper powder in 10mL concentrated nitric acid and diluted to 100mL with water in a calibrated flask. Working solutions were prepared by appropriate dilution of the stock solution.

Apparatus

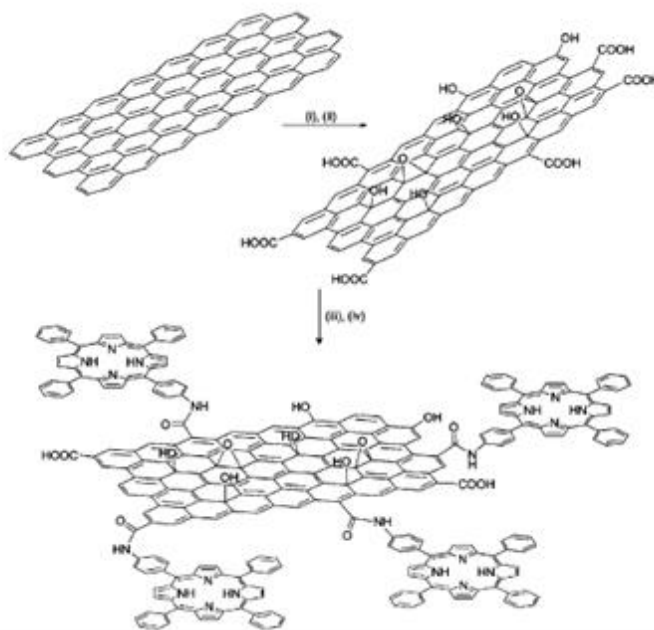
Determination of Cu²⁺ contents in working samples were carried out by a Varian spectra A.200 model atomic absorption spectrometer equipped with a high intensity

hallow cathode lamp(HI-HCl) according to the recommendations of the manufacturers. These characteristics are tabulated in(Table 1). olid phase extractions were carried out by glassy membrane disks, ENVI-18DISK™ 47mm diameter ×0.6 mm thickness containing octadecyl silica bonded phase (30 μm particles, 70 A° pore size) obtained from Supelco in conjunction with a standard Millipore 47 mm filtration apparatus equipped with a vacuum pump. The pH measurements were carried out by an ATC pH meter (EDT instruments, GP 353).

Synthetic procedures

Preparation of GO-H₂P

GO (15 mg) was stirred in 20 mL of oxalyl chloride at 80 °C for 24 h to activate the carboxylic units by forming the corresponding acyl chlorides. Then, the reaction mixture was evaporated to remove the excess oxalyl chloride and the brownish remaining solid (GO-COCl) was washed with anhydrous tetrahydrofuran (THF). After centrifugation, the resulting solid material was dried at room temperature under vacuum. For the covalent coupling between the free amino function of H₂P and the acyl chloride of GO, 15 mg of GO-COCl was treated under anaerobic, dry conditions with 7 mg of H₂P dissolved in 6 ml of dry THF at room temperature for 72 h. The hybrid material, namely GO-H₂P, was obtained as a brown-gray solidby filtration of the reaction mixture through 0.2 mm PTFE filter and the filtrate was sufficiently washed with methylene chloride (4× 20 ml) to remove non-reacted free H₂P and then with diethyl ether (2 × 20 mL) before being dried under vacuum.



Scheme 1 A schematic illustration for the preparation of GO with covalently linked H₂P. (i) H₂SO₄/HNO₃ (2 : 1 v/v), (ii) KClO₃, 96 h, (iii) (COCl)₂,80 °C, 24 h, (iv) 5-(4-aminophenyl)-10,15,20-triphenyl-21,23H-porphyrin, THF, r.t., 72 h.

Sample extraction

Extraction were performed with glassy membrane disks, ENVI-18 DISK™ 47mm diameter ×0.6 mm thickness containing octadecyl silica bonded phase (30 μm particles,

70 A° pore size) from Supelco. The disks were used in conjunctions with a standard Millipore 47mm filtration apparatus connected to water aspirator.⁴⁶

1) Sample Treatment:

The water samples were filtered through 45µm nylon filters. Sampling vessels were polyethylene bottles soaked in 1 mol.L⁻¹ HNO₃ overnight and rinsed twice with deionized water. The analysis must be done within 2 days of sample collection to limit the risk of interconversion of copper(II). Then, 5mL of methanol was added to a 90mL portion of each before analysis. The surface of the ENVI-18 DISK™ disks is not modified with GO-H₂P and therefore could not retain Cu²⁺ ions properly. Instead, 10 mg of GO-H₂P was dissolved in an appropriate volume of an organic solvent (5mL) miscible with water. The most suitable solvent under the experimental conditions was acetone. The GO-H₂P solution was added to aqueous solution of Cu²⁺ and the mixture was stirred gently.

Disk cleaning and conditioning:

A disk was placed in the apparatus and was washed with 10mL of methanol to remove all contaminants arising from the manufacturing process and the environment. Then, the disk was dried by passing air through it for several minutes. To insure optimal extraction of the analytes of interest, the disk was again washed with 10mL of methanol, immediately followed by 10mL of water, without letting the surface of the disk dry. This step pre-wets the disk surface prior to extraction. Improper performance of this step causes slow flow – rate and poor analyte recoveries. It is important to avoid any air contact with the surface of the disk before the addition of the sample.

Sample addition

After complete homogenization, accurate volumes of the sample solutions (100mL portions) were transferred to the top reservoir of the disk apparatus. At the same time, the solution was drawn through the disk by applying a mild vacuum. Application of vacuum was continued until the disk was completely dry (about 5 minute).

Analyte elution: In order to elute the analyte selectively, exactly 5 mL of acidified solvents 0.1M HCl in methanol was passed through the disk and collected into a 5.0 mL volumetric flask under the extraction funnel. It was found that ultrapure alcoholic organic solvents were the best eluting agents. The concentration of copper(II) in the eluates were then determined by FAAS using an external calibration graph.

Analysis of Sample paraffin-embedded tissues from Liver loggerhead turtles specimens

Selected areas from fresh frozen tissues from Liver loggerhead turtles specimens were sliced in three pieces (numbered as 1, 2 and 3) of approximately 10mm×5mm×2mm each. Sets of pieces of set 1 (controls), were placed into a vacuum chamber at 50 °C overnight to dry (until a constant weight was obtained), and the sets 2 and 3 were subjected to the standard 10% buffered formalin fixation and paraffin embedding [25] histological process using a tissue processor (Tissue-Tek VIP, Sakura Finetek USA Inc., Torrance, CA). After the paraffin embedding process, tissues were subsequently excised from the blocks with a titanium knife and deparaffinized in xylene at 55 °C for 1 h in the tissue processor (the set 2), or with hexane at

20 °C for 1 week with frequent changes of the solvent in handling-based procedure (the set 3). Xylene was of a grade routinely used for the FFPE process and hexane was of “Optima” grade (Fisher Scientific). Upon deparaffinization, the tissue samples were dried in a vacuum chamber until constant weight was obtained. Each dried sample (of the sets 1–3) was divided into three portions (5–10 mg each) to be further analyzed as triplicates.

3. Results and Discussion

The treatment of Graphene oxide with covalently linked porphyrin (GO-H₂P) can lead to the derivatization of both the edge carboxyl and surface hydroxyl functional groups via formation of amides [20] or carbamate esters [21], respectively. The formation of GO-H₂P was followed by ATR-IR spectroscopy. Initially, in the spectrum of GO, the carbonyl vibration appears at 1716 cm⁻¹, while there are fingerprints at 3616 cm⁻¹ and 3490 cm⁻¹ due to the presence of hydroxyl species at the basal plane of graphene. The covalent linkage of H₂P with the acyl chloride activated GO is evident from the presence of a band at 1630 cm⁻¹, which is characteristic for the carbonyl groups of the amide units [23] (see Fig. 2). The amount of porphyrin attached onto the graphene sheet was evaluated by thermogravimetric analysis. As compared with the TGA results of pure graphite, which is thermally stable up to 900 °C under nitrogen, and GO which decomposes above 600 °C, after having lost the oxygenated species at 240 °C (i.e. 14.7% weight loss), the 6% weight loss occurred in the temperature range 250–550 °C for the GO-H₂P material, is attributed to the decomposition of H₂P (Fig. 2). The GO-H₂P material forms a stable dispersion in DMF at a concentration not exceeding 1 mg mL⁻¹. The electronic absorption spectrum of GO-H₂P in DMF (Fig. 3), shows (i) a broad signal monotonically decreasing from the UV to the visible region, which is attributed to GO and (ii) a characteristic band at 420 nm (Soret-band) corresponding to the covalently grafted H₂P units (the Q-bands at 516, 557, 589 and 648 nm were flattened to the base line in the GO-H₂P material). Interestingly, the absorption of porphyrin in the GO-H₂P material is broadened, shortened and bathochromically shifted (ca. 2 nm) as compared to that of the free H₂P, a result that corroborates not only the linkage of porphyrin with the GO sheets but also electronic interactions between the two species (i.e. GO and H₂P) in the ground state. These results are in agreement with studies based on other hybrid systems consisting of porphyrins covalently grafted to carbon nanotubes and nanohorns [20].

3.1. Evaluation of the role of the ligand

Some preliminary experiments were performed for investigation of absence or presence of PAN on the quantitative extraction of copper(II). It was concluded that the membrane disk itself does not show any tendency for the retention of copper(II), but introduction of 100mL portions of aqueous copper(II) samples containing 10µg of copper(II) and 10mg of GO-H₂P leads to satisfactory its retention (Table 2).

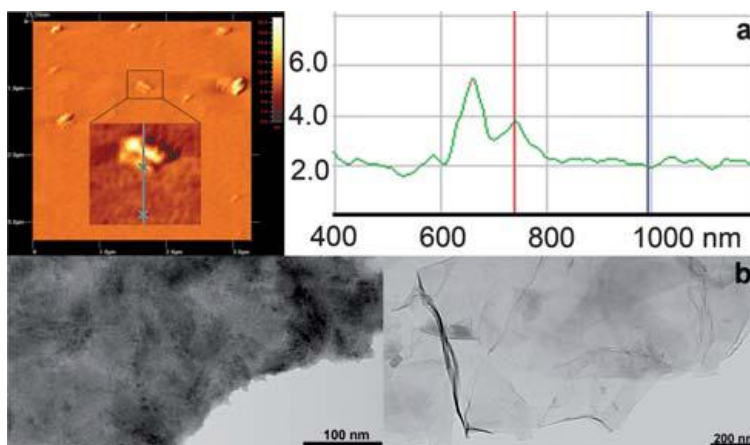


Fig. 1. (a) Representative AFM image of GO-H₂P and profile analysis showing a height of 1.77 nm for the enlarged region. Section analysis of other regions of the image show height ranges of 1.5–3.5 nm. (b) TEM images of the intact graphite (left panel) and GO-H₂P hybrid material (right panel)

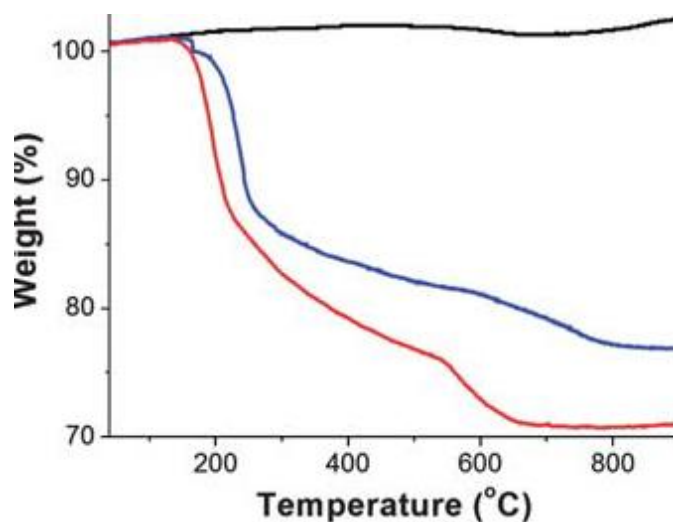


Fig. 2. The TGA graphs of graphite (black), GO (blue) and GO-H₂P (red), obtained under an inert atmosphere.

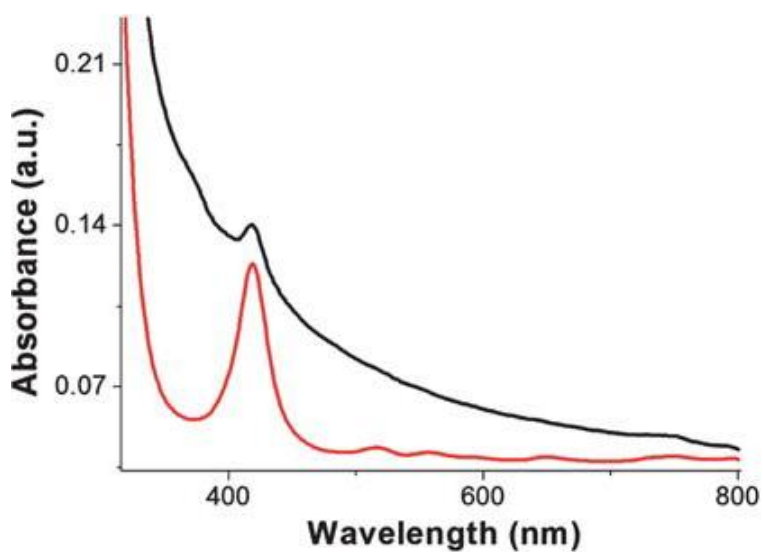


Fig. 3. The UV-vis spectra of GO-H₂P (black) and free H₂P (red), obtained in DMF.

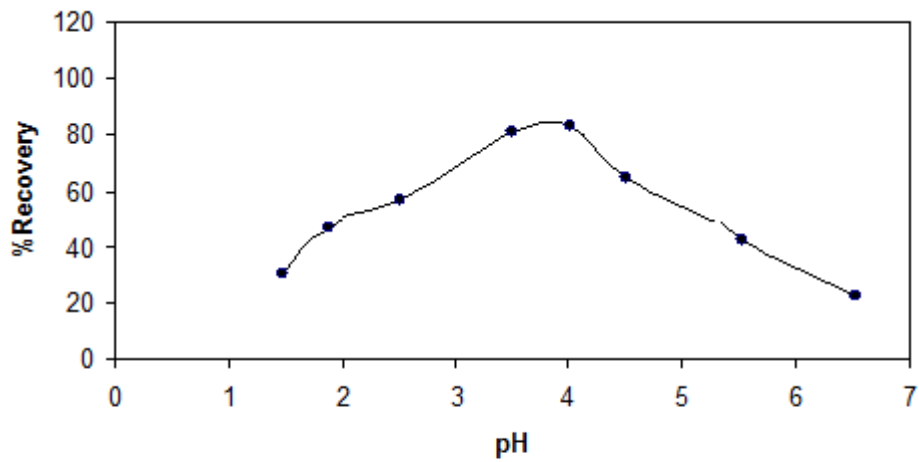


Fig. 4. Influence of sample pH and dissolving solvent of GO-H₂P on the percentage recovery of Cu(II).

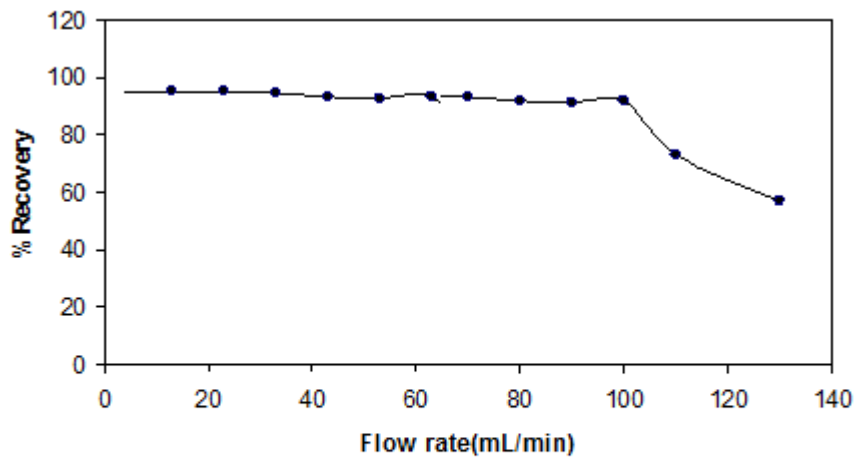


Fig. 5. The effect of the flow-rate on extraction percent of Cu(II).

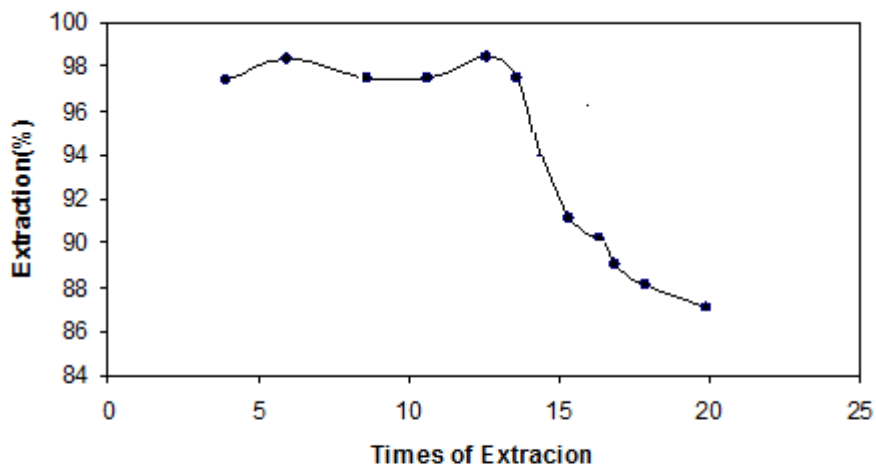


Fig. 6. Influence of eluent(5mL of methanol)type on disk efficiency.

Table 1. The operational conditions of flame for determination of copper

Slit width	0.7 nm
Operation current of HI-HCL	15 mA
Resonance fine	324.8nm
Type of background correction	Deuterium lamp
Type of flame	Air/acetylene
Air flow	7.0 mL.min ⁻¹
Acetylene flow	1.7 mL.min ⁻¹

Table 2. The effect of presence of GO–H₂P on extraction percent of copper(II)^a.

GO–H ₂ P	pH	Extraction percent of copper(II)
Absence	2-6	0.06(6.0) ^b
Presence	2-6	98.0(2.5) to 65(2.4)

^a Initial samples contained 10µg of copper(II) in 100mL of water.

^b Values in parentheses are RSD_s based on five individual replicate analyses.

Table 3. Effect of different eluting solvents on Percentage recovery of copper(II) adsorbed on the disk ^a

Stripping solution	% Recovery		
	2ml	5ml	10ml
Methanol	86(2.6) ^b	98.5(2.6)	98.4(2.0)
Acidified methanol ^c	57(2.3)	87.2(2.2)	83.5(1.7)
Ammoniacal methanol ^d	57 (2.5)	87.5(2.6)	86.9(2.2)
Ethanol	89.1(1.7)	100(1.5)	99.8(2.3)
Acetonitril	36(4.8)	46(5.5)	67(3.0)
Formic acid(1M)10% V.V ⁻¹ mthanol	55(1.2)	65(2.0)	78(2.0)
Hydrochloric acid(1M)10% V.V-1 mthanol	59.3(1.9)	92.4(2.4)	93s(2.8)
Hydrochloric acid(1M)10% V.V ⁻¹ mthanol	53(2.5)	85 (2.6)	93(1.8)
Nitric acid(2M)10% V.V ⁻¹ mthanol	54(1.9)	86(2.2)	87(2.0)
Nitric acid(1M)10% V.V ⁻¹ mthanol	65(2.5)	85(2.3)	89(1.7)
Ethanol	83(2.8)	95.7(2.0)	97.7(2.9)

^a Contained 10 µg of each copper in 100 mL water.

^b Values in parentheses are RSDs based on five individual replicate analysis.

^c Acidified solvents obtained by addition of 0.1M HCl.

^d Ammoniacal solvents obtained by addition of 0.1M NH₃

Table 4. Percent recovery of copper from the modified membrane disk in the presence of 0.01 M of different counter anions ^a.

counter anion	% Recovery
Cl ⁻	23.5
Br ⁻	22.7
ClO ₄ ⁻	33.8
SCN ⁻	45.6
Picrate	74.9
Acetate	97.5

^a Initial samples contained 10µg of copper(II) in 100mL of water.

Table 5. Influence of the GO–H₂P amount on the recovery of Cu(II) ions ^a.

GO–H ₂ P amount (mg)	Recovery(%) of Cu(II)
2	37.5(2.2) ^b
5	48.6(2.9)
8	83.8(2.7)
10	96.9(2.9)
15	98.9(2.6)
20	99.7(2.5)

^a Initial samples contained 10 µg of each copper in 100 mL water.

^b Values in parentheses are RSDs based on five individual replicate analysis.

Table 6. Separation of copper from binary mixtures ^a

Diverse ion	Amounts taken(mg)	% Found	%Recovery of Cu ²⁺ ion
Na ⁺	92.4	1.15(2.4) ^b	98.7(1.8)
K ⁺	92.5	1.32(2.3)	98.5(2.9)
Mg ²⁺	14.5	0.7(1.2)	98.9(1.8)
Ca ²⁺	26.3	2.25(3.0)	98.5(1.6)
Sr ²⁺	2.45	2.85(2.5)	98.4(2.0)
Ba ²⁺	2.66	3.16(2.1)	98.3(2.3)
Mn ²⁺	2.66	1.75(2.2)	97.3(2.8)
Co ²⁺	2.16	1.4(2.3)	99.1(2.9)
Ni ²⁺	1.65	2.0(2.4)	98.5(2.6)
Zn ²⁺	2.78	1.97(2.1)	98.4(2.2)
Cd ²⁺	2.55	1.92(0)	98.2(2.8)
Pb ²⁺	0.54	2.7(1.9)	97(2.7)
Hg ²⁺	0.44	2.81(2.1)	97.7(2.8)
Ag ⁺	2.63	3.45(2.9)	96.6(2.9)
Cr ³⁺	1.73	2.92(2.30)	97.3(2.4)
UO ²⁺	2.84	2.8(2.1)	98.3(2.7)

^a Initial samples contained 10µg Cu²⁺ and different amounts of various ions in 100 mL water(0.1 M acetate ion).

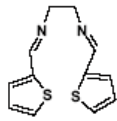
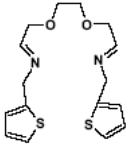
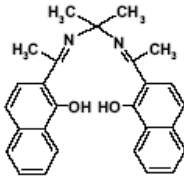
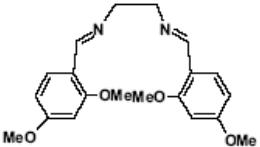
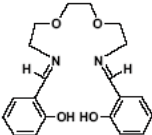
^b Values in parentheses are RSDs based on five individual replicate analysis.

Table 7. Recovery of copper added to 1000mL of different water samples (containing 0.1Macetate at pH= 4.0-4.5).

Sample	Cu ²⁺ added (µg)	Cu ²⁺ determined(ng.mL ⁻¹)	ICP-AES
Tap water	0.0 10.0	1.74(1.4) ^a 11.98(3.2)	ND 11.7
Snow water	0.0 10.0	4.45(2.4) 14.97(2.0)	ND 14.7
Rain water	0.0 10.0	2.65(2.3) 12.75(2.4)	ND 12.3
Sea Water	0.0 10.0	12.64(2.3) 22.93(2.0)	12.5 23.1
Development of a methodology for the determination of Cu in FFPE tissue	0.0 10.0	- 10.02(1.9)	

^a Values in parentheses are %RSDs based on five individual replicate analysis; ^b Not detected.

Table 8. The effect of the different Schiff's base molecular structures on their selectivity toward different metal traces by using C₁₈ octadecyl silica bonded phase sorbent.

Schiff's base	Ion(s) of interest	Ref.
	Pb ⁺²	23
	Pb ⁺²	59
	Cu ⁺²	25
	Cu ⁺² & Pb ⁺²	61
	Cu ⁺² & Pb ⁺²	62

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