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Extraction sorbent with octadecane-functionalized nano graphene for the preconcentration of Cu(II) in water and tissues from liver loggerhead turtles specimens

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ABSTRACT: A simple, highly sensitive, accurate and selective method for determination of trace amounts of Cu^{2+} in water samples was reported. In this paper, Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl] ethanethioate (IIDE) in order to prepare an effective sorbent for the preconcentration and determination of Cu. The sorption capacity of Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl]ethanethioate (IIDE)-modified Octadecane-functionalized nano graphene (OD-G) (IIDE MS) was 82.34 $mg.g^{-1}$ and the optimum pH for the quantitative recovery of Cu was found as 5.3. The optimum flow rate, sorbent amount and sample volume were 8 $mL.min^{-1}$, 300 mg and 50 mL, respectively. 10 mL of 0.1 $mol.L^{-1}$ HCl was the most suitable eluent. The recommended method is simple and reliable for the determination of Cu without any notable matrix effect and successfully applied to environmental water samples. The limit of detection of the proposed method is 7.5 ng per mL. The method was applied to the extraction and recovery of Cu(II) in different water samples. 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. This method exhibits the superiority in compared to the other adsorption reagents because of the fact that there is no necessity of any complexing reagent and optimum pH of solution presents in acidic media. In this method is relative standard deviation (R.S.D.) of 2.7%.

Keywords: *Cu; Formalin-fixed paraffin-embedded (FFPE); Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl]ethanethioate (IIDE) -modified silica-gel; Octadecane-functionalized graphene (OD-G); Tissues from Liver loggerhead turtles*

1. INTRODUCTION

Copper, at trace concentrations, acts as both a micronutrient and a toxicant in marine and fresh water systems (Leyden, *et al.*, 1976; Narin, *et al.*, 2000; Akama, *et al.*, 2000; Ohta, *et al.*, 2001; Cuculic, *et al.*, 1997; Moghimi, *et al.*, 2012).The direct determination of trace metals especially toxic metal ions such as Cu, tin, arsenic, lead, antimony and selenium from various samples requires mostly an initial and efficient pre-concentration step (Leyden, *et al.*, 1976). This pre-concentration is required to meet the detection limits as well as to determine the lower concentration levels of the analyte of interest (Jones, *et al.*, 1983). This can be performed simply in many ways including liquid and solid phase extraction techniques (Nambiar, *et al.*, 1998; Caroli, *et*

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al., 1991). The application of solid phase extraction technique for pre- concentration of trace metals from different samples results in several advantages such as the minimal waste generation, reduction of sample matrix effects as well as sorption of the target species on the solid surface in a more stable chemical form(Alexandrova, *et al*., 1993).

The normal and selective solid phase extractors are those derived from the immobilization of the organic compounds on the surface of solid supports which are mainly polyurethane foams (Arpadjan, *et al*., 1997), filter paper (Leyden, *et al*., 1975), cellulose (Gennaro, *et al*., 1983) and ion exchange resins (Grote, *et al*., 1985). Silica gel, alumina, magnesia and zirconia are the major inorganic solid matrices used to immobilize the target organic modifiers on their surfaces (Unger 1979) of which silica gel is the most widely used solid support due to the well documented thermal, chemical and mechanical stability properties compared to other organic and inorganic solid supports (Boudreau, *et al*., 1989). The surface of silica gel is characterized by the presence of silanol groups, which are known as weak ion exchangers, causing low interaction, binding and extraction of the target analytes (Kvitek, *et al*., 1982). For this reason, modification of the silica gel surface with certain functional groups has successfully been employed to produce the solid phase with certain selectivity characters (Bruening, *et al*., 1991).

Two approaches are known for loading the surface of solid phases with certain organic compounds and these are defined as the chemical immobilization which is based on chemical bond formation between the silica gel surface groups and those of the organic modifier, and the other approach is known as the physical adsorption in which direct adsorption of the organic modifier with the active silanol groups takes place (Unger 1979). Selective solid phase extractors and pre-concentrators are mainly based on impregnation of the solid surface with certain donor atoms such as oxygen, nitrogen and sulfur containing compounds (Mahmoud 1997; Mahmoud, *et al*., 1997; Tong, *et al*., 1990; Dadler, *et al*., 1987). The most successful selective solid phases for soft metal ions are sulfur-containing compounds, which are widely used in different analytical fields. Amongst these sulfur-containing compounds are dithiocarbamate derivatives for selective extraction of Cu(II) (Mahmoud 1998; Mahmoud 1999) and pre-concentration of various cations (Leyden, *et al*., 1976; Narin, *et al*., 2000; Akama, *et al*., 2000; Ohta, *et al*., 2001; Cuculic, *et al*., 1997; Moghimi, *et al*., 2009; Thurman 1998; Pawliszyn 1997; Izatt, *et al*., 1996; Hagen, *et al*., 1990; Krueger 1995; Yamini, *et al*., 1994; Shamsipur, *et al*., 1999; Shamsipur, *et al*., 2001; Brunner, *et al*., 2003; Zelder, *et al*., 2004; Boll, *et al*., 2005; Nayebi, *et al*., 2006; Moghimi, *et al*., 2007; Moghimi 2007) and 2-mercaptobenzothiazol-modified silica gel for on-line pre-concentration and separation of silver for atomic absorption spectrometric determinations (Qiaosheng, *et al*., 1998). Ammonium hexa-hydroazepin-1-dithiocarboxylate (HMDC)-loaded on silica gel as solid phase pre-concentration column for atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) was reported (Alexandrova, *et al*., 1993). Mercapto-modified silica gel phase was used in pre-concentration of some trace metals from seawater (Moghimi, *et al*., 2010). Sorption of Cu(II) by some sulfur containing complexing agents loaded on various solid supports (Tajodini, *et al*., 2010) was also reported. 2-Amino-1-cyclopentene-1-dithiocaboxylic acid (ACDA) for the extraction of silver(I), Cu(II) and palladium(II) (Moghimi, *et al*., 2009), 2-[2-triethoxysilyl-ethylthio] aniline for the selective extraction and separation of palladium from other interfering metal ions (Narin, *et al*., 2000) as well as thiosemicarbazide for sorption of different metal ions (Campderros, *et al*., 1998) and thioanilide loaded on silica gel for pre-concentration of palladium(II) from water (Narin, *et al*., 2000) are also sulfur contaning silica gel phases.

This study focuses on the utilization of IIDEMS as an efficient octadecane-functionalized nano graphene (OD-G) in the preconcentration step of Cu(II) ions prior to FAAS determination. The influences of some analytical conditions on the preconcentration procedure, such as initial pH, sample volume, eluent type and volume, sorbent amount, flow rate were investigated.

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(II).

EXPERIMENTAL

Apparatus

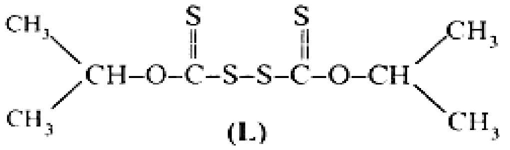
Determination of Cu^{2+} contents in working samples were carried out by a Varian spectra A.200 model atomic absorption spectrometer equipped with a high intensity hallow cathode lamp(HI-HCl) according to the recommendations of the manufacturers. Instrumental parameters were selected according to the manufacturer's suggestion. The infrared spectra of the materials were recorded on a Perkin Elmer spectra 100-IR spectrometer (Waltham, MA, USA) using KBr disk in the range of 4000-400 cm^{-1}. Heidolph PD 5201 (Schwabach, Germany) model peristaltic pump with eight heads was used for controlling the flow of the liquid into column. The pH measurements were carried out by an ATC pH meter (EDT instruments, GP 353).

Reagents

Octadecane-functionalized nano graphene (OD-G) was used as the support material. All the chemicals used in this study were of analytical grade deionized water was used in all experiments. The standard solution of Cu(II) (1000 $mg.L^{-1}$) for the calibration of AAS was purchased from Merck (Darmstadt, Germany). The other concentrations of the standard solutions were prepared by diluting this solution. A stock solution of Cu(II) was prepared by dissolving appropriate amount of $Cu(NO_3)_2$ (Merck) in doubly distilled deionized water and the other concentrations of the working solutions were obtained by diluting this stock metal solution. The pH of the solutions was adjusted to desired values with 0.1 M HCl and/or 0.1 M NaOH solutions. Working solutions were prepared by appropriate dilution of the stock solution.

Synthesis of Molecular structure of Isopropyl 2-[(isopropoxycarbothioyl) disulfanyl] ethanethioate

Iodine (1 mmol) in CH_2Cl_2 (10 mL) was added to a stirred solution of potassium o-isopropyl (dithiocarbomate) (1 mmol) in CH_2Cl_2 (10 mL) and stirred for 1 h. The reaction mixture was washed with 10% aqueous $Na_2S_2O_3$ (2×10 mL) and H_2O (2×10 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. More purification carried out with the re-crystallization in hexane so that pale yellow crystals of L were obtained in 90% yield (0.24 g). The structure and purity of L was confirmed by elements analysis, NMR and IR Spectroscopy. ^1H NMR (CCl_4), δ (ppm): 1.43 (t, 12H, CH3), 5.63 (m, 2H, CH). IR (KBr). νmax (cm^{-1}): 2979.8 (s), 2869.9 (w), 1463.9 (s), 1442.7 (s), 1373.0 (s), 1271.1 (s, b), 1145.6 (s), 1082.2 (s), 1048.0 (s, b), 898.8 (s), 796.5 (s), 690.5 (m) (Scheme 1).

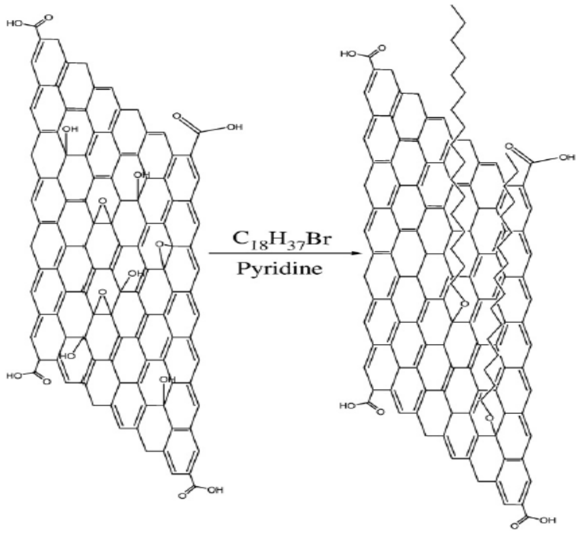


(L)

Scheme 1: Molecular structure of Isopropyl 2-[(isopropoxycarbothioyl)disulfanyl]ethanethioate. (Harvey *et.al.*, 1950)

Synthesis of octadecane-functionalized graphene (OD-G)

The GO was synthesized according to the modification of Hummers' methods (Hummers, *et al.*, 1958). In a typical preparation of OD-G, 50 mg of GO and 100 mL of dimethylformamide (DMF) were added to the flask with sonication for 1 hour to get a homogeneous dispersion. Five hundred milligrams BOD and 30 ml pyridine were added into the reaction mixture. The



Scheme 2: Schematic drawing of the reaction system for the synthesis of OD-G, with ethanol and acetone twice, separately. The as-prepared OD-G was then dissolved in tetrahydrofuran (THF) or dichlorobenzene (DCB) by sonication for 30minutes (Liu, *et al.*, 2010)

mixture was then heated to 115°C and kept refluxing for 24 hours. To purify the OD-G, 100 ml ethanol was added to the mixture, followed by filtration with 0.45 µm PTFE membranes. The filter cake was washed.

Preparation of Isopropyl 2-[(isopropoxy carbothioyl) disulfanyl]ethanethioate modified Octadecane-functionalized nano graphene (OD-G)

All acids were of the highest purity available from Merck and were used as received. Methanol and Chloroform were of HPLC grade from Merck. Analytical grade nitrate salts of lithium, sodium, potassium, magnesium, calcium, strontium, barium, zinc, cadmium, copper(II) nickel, cobalt(II), and Cu (II) were of the highest purity. Ultra pure organic solvents were obtained from E. Merck, Darmstat, Germany. The stock standard solution of Cu (II) was prepared by dissolving 0.1000 g of the Cu(II) powder in 10 mL concentrated nitric acid and diluted to 1000 mL with water in a calibrated flask. Working solutions were prepared by appropriate dilution of the stock solution. In order to prepare a 0.1% Isopropyl 2-[(isopropoxy carbothioyl) disulfanyl] ethanethioate (IIDE) solution, 0.1 g of the reagent was dissolved in 10 mL of acetone and 8 mL of concentrated ammonia solution was added. The final volume of this solution was diluted to 100 mL with water. 100 mL of reagent solution was added into four grams of Octadecane-functionalized nano graphene (OD-G) suspended in 100 mL water and then mixed on a magnetic stirrer for 24 h. The final product was filtered, washed with doubly distilled deionized water and then dried at 100°C in an oven overnight.

Column preparation

A glass column (5.5 cm height ×9 mm i.d.) was packed with a known amount of IIDEMS between two layers of glass wool into the column. A definite volume of Cu(II) solution (1 µg mL^{-1}) was passed through the column. All the column studies were performed at room temperature of 25°C.

Preconcentration procedure

The preconcentration method was tested with synthetic Cu(II) solutions prior to its application to the real samples. For this purpose, an aliquot of 50 mL of standard Cu(II) solution (1 µg.mL^{-1}) was taken and the pH of the solution was adjusted to desired value with HCl and/or NH_3. The model solution was pumped through the column at a flow rate of 8 mL min^{-1} controlled with a peristaltic pump. The bound metal ions were eluted from IIDEMS with 10 mL of 0.1 mol L^{-1} HCl solution. The Cu(II) concentration in the eluate was determined by FAAS. The recoveries of Cu(II) were calculated from the ratio of concentration found by FAAS to that calculated theoretically. The general preconcentration procedure described above was carried out to optimize the experimental conditions such as pH, amount of adsorbent, flow rate, type, concentration and volume of the elution solutions, etc.

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 10 mm × 5 mm × 2 mm 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 embedding31 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.

RESULTS AND DISCUSSION

Characteristics of the material

The morphologies of GO and OD-G are observed by TEM. As shown in Fig. 1a, the image of GO sheets is

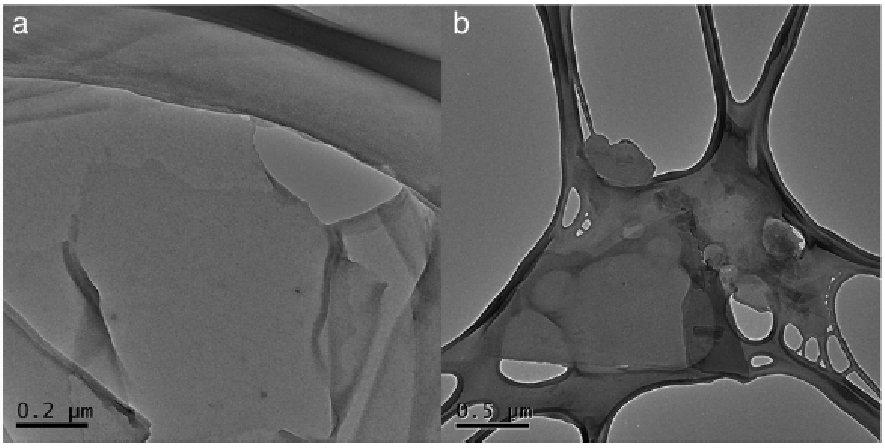
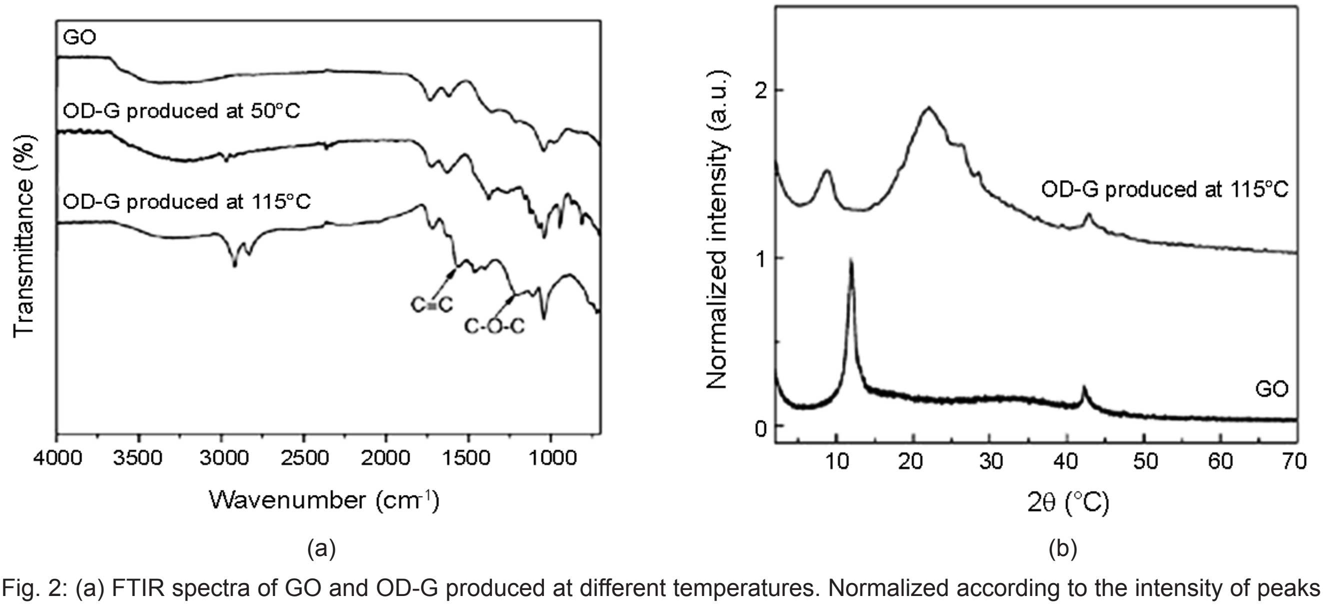


Fig. 1: TEM images of (a) GO sheets and (b) OD-G single sheets. (Saitoh, *et al.*, 2004)

smooth with the average size of about 1 μm. The verge is quite clear while some tend to fold and roll. After functionalized with OD groups, the size of OD-G is about 0.5 μm, which is smaller than that of the original GO (Fig. 1b). During the functionalization, the reaction between oxygen groups from GO and BOD could split the larger graphene into smaller graphene sheets. Similar results were reported by (Sun, *et al.*, 2010; Graf, *et al.*, 2007). The introduction of OD groups can effectively prevent the aggregation of GO during reduction. The FTIR spectra are tested for confirming the effective reduction and ether-functionalization with OD groups of GO. From Fig. 2a, the FTIR spectrum of OD-G presents the doublet bands at 2854 and 2923 cm^{-1}, which are attributed to the antisymmetric and symmetric C-H stretching vibrations of the -CH_2- groups from OD groups (Tuzen, *et al.*, 2009). The band centered at 1200 cm^{-1} is resulted from the C-O-C asymmetric stretch (Moghimi, *et al.*, 2012; Choi, *et al.*, 2003) this suggests the ether-functionalization occurs between GO and BOD. In addition, the band at 1574 cm^{-1} is connected to the C=C skeletal vibration of reduced graphene sheets (Choi, *et al.*, 2003) .This indicates that GO has been effectively reduced during the functionalization process in pyridine at 115°C. (McAllister, *et al.*, 2007) reported the deoxygenation by the nucleophilic substitution between epoxy groups of GO and alkylamine or diaminoalkane. So



(a) (b)

Fig. 2: (a) FTIR spectra of GO and OD-G produced at different temperatures. Normalized according to the intensity of peaks at 1725 nm, (b) XRD patterns of GO and OD-G produced at 115°C (Saitoh, *et al.*, 2004)

we speculate that the reduction is associated with the nucleophilic attack of GO by pyridine. Meanwhile, for the FTIR spectra of OD-G at 50°C, the lack of bands at 1574 cm^{-1}, 2854 cm^{-1} and 2923 cm^{-1} suggests that GO is neither functionalized with OD groups nor reduced by pyridine at this temperature. Fig. 2b shows the XRD patterns of GO and OD-G produced at 115°C. It can be seen that the XRD pattern of OD-G produced at 115°C shows a broader peak of graphitic {002} diffraction plane at $2\theta = 21.95°$ which is resulted from the disordered stacking of reduced graphene sheets (Saitoh, *et al.*, 2004).This further confirms the effective reduction during the ether-functionalization. Additionally, the weak peak at $2\theta = 8.76°$ is associated with the {001} diffraction plane of OD-G. The corresponding interlayer spacing is 10.1 Å, which is larger than that of original GO due to the successful ether-functionalization with OD groups.

Effect of pH on the recovery of Cu

The pH of the sample solutions were adjusted to different values between 2-8, by addition of hydrochloric acid or a suitable buffer such as sodium acceate-acetic acid or sodium dihydrogen phosphate- disodium hydrogen phosphate, and then solutions passed through the column. Eventually, the metal ions were stripped by 5 mL of HNO_3, 1M followed by flame atomic absorption determination of the eluted Cu(II). Then, percentage recovery at various pH values was determined (Fig. 3). According to the results shown in Fig. 3 up to pH 4.5-5.5, complete recoveries are obtained. However, at higher pH values, percentage recovery decreases. This is due to fact that in an acidic solution the protonation of Isopropyl 2- (isopropoxycarbothioyl)disulfanyl]ethanethioate (IIDE) occurs and there is a weak tendency for retention between Cu(II)and 1-nitroso-2-naphthol-3,6-disulfonic acid, whereas at higher values (pH>5.7), Cu(II) reacts with hydroxide ions to produce $Cu(OH)_2$. Therefore, sodium acceate-acetic acid buffer with pH = 5.3 was used for the preconcentration step. Other solvents used for dissolving Isopropyl 2-(isopropoxycarbothioyl)disulfanyl]ethanethioate (IIDE) were 5 mL of HNO_3 (1M). The influences of these solvents on the recoveries as a function of pH are compared and shown in Fig. 3.

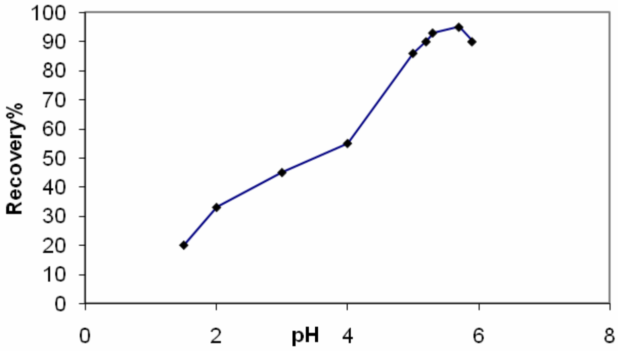


Fig. 3: Effect of pH on the recovery of Cu (II) by IIDEMS

Evaluation of the role of the ligand

Some preliminary experiments were performed for investigation of absence or presence of Isopropyl 2-(isopropoxycarbothioyl)disulfanyl]ethanethioate (IIDE) on the quantitative extraction of Cu(II). It was concluded that the surface itself does not show any tendency for the retention of Cu(II), but introduction of 100 mL portions of aqueous Cu(II) samples containing 10 µg of Cu(II) and 10 mg of Isopropyl 2-(isopropoxycarbothioyl)disulfanyl]ethanethioate (IIDE) Cus to satisfactory its retention (Table 1). The latter case is most probably attributed to the existence of a considerable interaction between Cu(II) and the Isopropyl 2-(isopropoxycarbothioyl)disulfanyl]ethanethioate (IIDE). It should be mentioned that formation of stable complexes between Cu(II) and Isopropyl 2-(isopropoxycarbothioyl)disulfanyl] ethanethioate (IIDE) at pH 5.3 is probably due to an ion pair formation mechanism. However, at pH higher than 5 the retention and percentage recovery of Cu(II) are negligible.

Choice of eluent

In order to select the most appropriate eluent for the quantitative stripping of the retained Cu(II) on the column, 5 mL of various non organic solvents were tested. The results can be seen, the best eluting solvents

Table 1: The effect of presence of IIDE on extraction percent of Cu (II)[a]

IIDE	pH	Extraction percent of Cu (II)
Absence	2-6	0.07(6.6)[b]
Presence	2-6	98.8(2.9) to 65(2.0)

(a) Initial samples contained 10 µg of Cu (II) in 100 mL of water; (b) Values in parentheses are RSDS based on five individual replicate analyses

Table 2: Percent recovery of Cu(II) from the modified of Octadecane-functionalized nano graphene (OD-G) in the presence of 0.01 M of different counter anions[a]

Counter anion	Recovery (%)
Cl^-	28.8
`Br^-	22.0
ClO_4^-	30.6
SCN^-	42.8
Picrate	74.7
Acetate	92.9

were found to be 5 mL of 0.1 mol.L^{-1} HCl, resulting in quantitative elution of Cu(II) from the column.

Effect amount of counter anion

In order to investigate the effect of counter ion on the recovery Cu(II) ions by the modified column, different counter anions were tested Table 2, it is immediately obvious that the nature of the counter anion strongly influences the retention of Cu(II) ions by the column. The results revealed that the Isopropyl 2-(isopropoxy-carbothioyl)disulfanyl]ethanethioate (IIDE) behaves as a neutral ionophore in the pH range 4.5-5.3 components (Moghimi, *et al.*, 2012; Choi, *et al.*, 2003; Kaiss, *et al.*, 2007) so that the Cu(II) ions are retained as ion pair complexes by the column. As seen, acetate ion is the most efficient counter anion for the SPE of Cu(II) ions. The influence of the concentration of sodium acetate ion on Cu(II) recovery was investigated, and the results are shown in Table 2. As seen, the percent recovery of Cu(II) increased with the acetate concentration until a reagent concentration of about 0.1 M is reached, beyond which the recovery remained quantitative. Moreover, acetate ion acts as a suitable buffering agent, while it effectively contributes to the ions-pair formation; thus, in the SPE experiments, there was no need for the addition of any buffer solution.

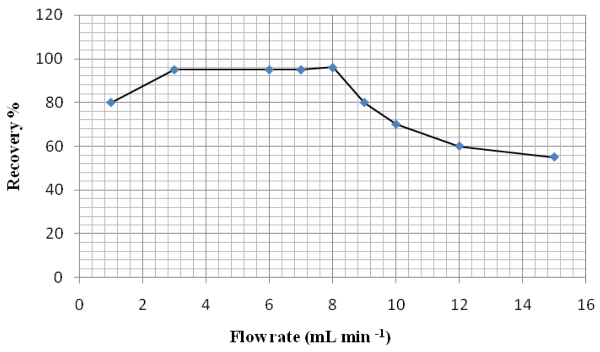


Fig. 4: Effect of flow-rate of sample solutions on the recovery of Cu (II) by IIDEMS

The influence of flow-rate

One of the most important parameters affecting solid phase extraction is the speed of the process. Hence, the effect of flow-rates on extraction efficiencies was investigated. It was found that in the range of 1-15 mL.min^{-1}, the retention of Cu(II) was not considerably affected by the sample solutions flow-rates and Cu to reproducible and satisfactory results (Fig. 4). Thus, the flow-rate was maintained at 8.0 mL.min^{-1} throughout the experiment.

Quantity of the Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl]ethanethioate (IIDE)

The optimum amount of Isopropyl 2-(isopropoxy-carbothioyl)disulfanyl]ethanethioate (IIDE) for the quantitative extraction of Cu(II) was also investigated by adding various amounts of it to solution (between 2-20 mg). The results are listed in Table 3. The experimental results revealed that the extraction of Cu(II) was quantitative using a sample solution containing more than 10 mg Isopropyl 2-(isopropoxycarbothioyl) disulfanyl]ethanethioate (IIDE). Hence, subsequent extractions were performed with 15 mg of Isopropyl 2-(isopropoxycarbothioyl)disulfanyl] ethanethioate (IIDE).

Table 3: Influence of the IIDE amount on the recovery of Cu (II) ions[a]

IIDE amount (mg)	Recovery (%) of Cu (II)
2	30(2.7)[b]
5	45(2.6)
8	80(2.5)
10	95.8(2.4)
15	99.0(2.5)
20	98.1(2. 4)

(a) Initial samples contained 10 μg of each Cu (II) in 100 mL water; (b) Values in parentheses are RSDs based on five individual replicate analysis

Table 4: Separation of Cu (II) from binary mixtures [a]

Diverse ion	Amounts taken(mg)	Found (%)	Recovery of Cu^{2+} ion (%)
Na^+	92.0	1.19(2.6)[b]	98.9(1.9)
K^+	92.9	1.30(2.0)	98.9(2.1)
Mg^{2+}	14.2	0.62(1.8)	99.2(2.0)
Ca^{2+}	20.3	2.20(2.0)	98.5(2.7)
Sr^{2+}	2.80	2.87(2.2)	98.4(2.0)
Ba^{2+}	2.90	3.15(2.3)	98.3(2.8)
Mn^{2+}	2.26	1.73(2.5)	97.4(2.8)
Co^{2+}	2.33	1.20(2.7)	98.8(2.9)
Ni^{2+}	1.90	2.13(2.4)	98.0(2.4)
Zn^{2+}	2.10	1.74(2.0)	98.6(2.2)
Cd^{2+}	2.35	1.95(2.3)	98.2(2.6)
Pb^{2+}	1.90	2.75(1.4)	97.7 (2.5)
Hg^{2+}	0.60	2.81(2.9)	97.7(2.4)
Ag^+	2.45	3.43(2.9)	96.6(2.5)
Cr^{3+}	1.70	2.93(2.1)	97.8(2.6)
UO^{2+}	2.60	2.82(2.2)	98.9(2.0)

(a) Initial samples contained 10μg Cu^{2+} 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

Analytical Performance

When solutions of 10 μg Cu(II) in 10, 50, 100, 500, and 1000 mL solutions under optimal experimental conditions were passed through the column, the Cu(II) was quantitatively retained in all cases. Thus, the breakthrough volume for the method must be greater than 1000 mL, providing a concentration factor of > 200. The limit of detection (LOD) of the method for the determination of Cu(II) was studied under the optimal experimental conditions. The LOD based on 3σ of the blank is 7.5 ng per mL. In order to investigate the selective separation and determination of Cu(II) ions from its binary mixtures with various metal ions, an aliquot of aqueous solutions (50 mL) containing 10 μg Cu(II) and mg amounts of other cations was taken and the recommended procedure was followed. The results are summarized in Table 4. The results show that the Cu(II) ions in binary mixtures are retained almost completely by the modified column, even in the presence of up to about 100 mg of various ions. Meanwhile, retention of other cations by the column is very low and they can be separated effectively from the Cu(II) ion. It is interesting to note that, in other experiments, we found that in the presence of high enough concentrations $NH_2OH.HCl$ as a suitable reducing agent (> 0.5M) (McAllister, 2007).

Analysis of water samples

To assess the applicability of the method to real samples, it was applied to the extraction and determination of Cu(II) from different water samples. Tap water (Tehran, taken after 10 min operation of the tap), rain water (Tehran, 25 January, 2015), Snow water (Varamin, 30 February, 2015) and Sea water (taken from Caspian Sea, near the Mahmoud-Abad shore) samples were analyzed. Development of a methodology for the determination of Cu(II) in FFPE tissue was performed in a number of steps to optimize the major factors affecting the precision of the analysis (Table 5). As can be seen from Table 5 the added Cu(II) ions can be quantitatively recovered from the water samples used.

Effect of the type, concentration and volume of the elution solutions

In order to determine the most suitable solution for the elution of Cu, three different types of eluting agents

Table 5: Recovery of Cu(II) added to 1000 mL of different water samples (contaning 0.1Macetate at pH = 5.3)

Sample	Cu^{2+} added (µg)	Cu^{2+} determined ($ng.mL^{-1}$)	ICP-AES
Tap water	0.0	1.74(2.0)[a]	ND[b]
	10.0	11.95(2.6)	11.6
Snow water	0.0	4.84(2.1)	ND
	10.0	14.96(2.4)	14.6
Rain water	0.0	2.65(2.3)	ND
	10.0	12.46(2.3)	12.3
Sea Water	0.0	12.65(2.4)	12.4
	10.0	22.96(2.0)	23.1
Development of a methodology for the determination of Cu^{2+} in FFPE tissue	0.0	N.D[b]	N.D[b]
	10.0	9.95(2.2)	2.5))10.05

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

(HCl, HNO_3 and EDTA) were evaluated. HCl was found to be most effective eluent (Table 6). 0.1 $mol.L^{-1}$ and 0.2 $mol.L^{-1}$ of HCl solution give quantitative recoveries (≥ 99%). Various volumes of 0.1 $mol.L^{-1}$ HCl were also examined as eluent and the results were represented in Figure 5. Since quantitative recovery (≥99%) was obtained with 10 mL of 0.1 $mol.L^{-1}$ HCl solution, it was selected as an eluent for the recovery of Cu by Isopropyl 2-[(isopropoxy carbothioyl) disulfanyl]ethanethioate (IIDE)-modified silica-gel (IIDEMS).

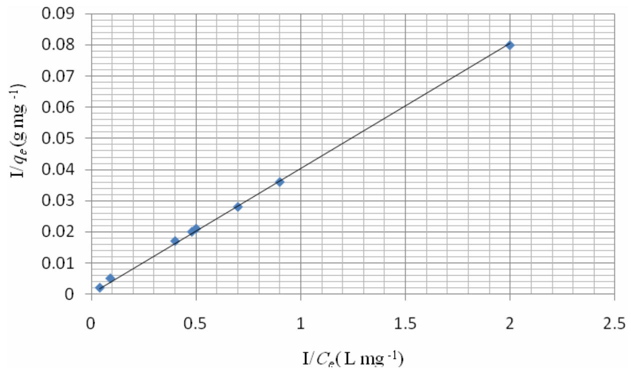


Fig. 6: Langmuir isotherm plot for the sorption of Cu (II) by IIDEMS

Adsorption capacity

Adsorption capacity of IIDEMS for Cu(II) ions was determined by a batch method. The initial Cu(II) ion concentration was changed from 100 to 1000 $mg.L^{-1}$ for the investigation of adsorption capacity of modified adsorbent. The pH of 50 mL of Cu(II) solution was adjusted to optimum value and then 300 mg of

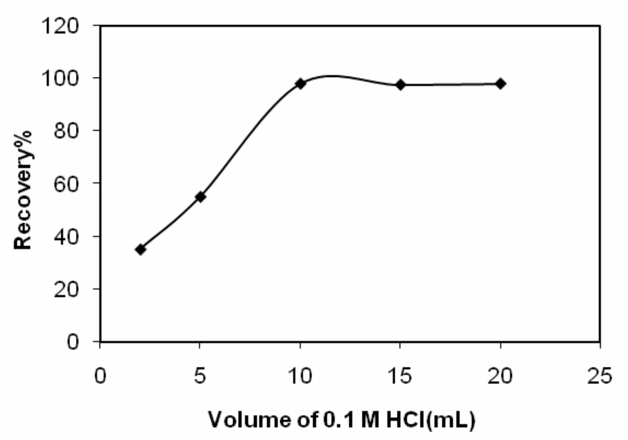


Fig. 5: Effect of the volume of 0.1 mol L^{-1} HCl solution on the recovery of Cu (II) by IIDEMS

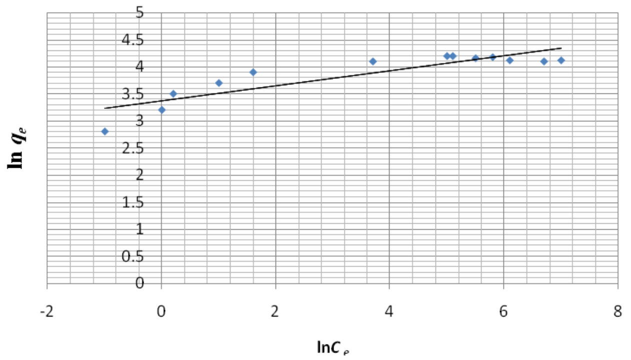


Fig. 7: Freundlich isotherm plot for the sorption of Cu (II) by IIDEMS

modified sorbent was added to Cu(II) solutions and mixed on a digitally controlled magnetic stirrer at a rate of 200 rpm. Suspended solids were separated from the adsorption medium by centrifugation at 4500 rpm for 3 min and Cu(II) ion concentrations were then measured using FAAS. The equilibrium data were analyzed by Langmuir (Saitoh, *et al.*, 2004) and Freundlich (McAllister 2007) isotherm models.

$$\text{Langmuir equation}\ \frac{1}{q_e} = \frac{1}{q_{max}} + \left(\frac{1}{q_{max}K_L}\right)\frac{1}{C_e} \qquad (1)$$

$$\text{Freundlich equation}\ \ln q_e = \ln K_F + 1/n \ln C_e \qquad (2)$$

Where qe and qmax are the equilibrium and monolayer sorption capacities of the sorbent (mg.g^{-1}), respectively, Ce is the equilibrium metal ion concentration in the solution (mg.L^{-1}) and KL is the equilibrium constant (L.mg^{-1}) related to the free energy of biosorption. KF (L.g^{-1}) and n (dimensionless) are Freundlich sorption isotherm constants. The Langmuir and Freundlich isotherm plots are shown in Figures 6 and 7, respectively and the model constants are presented in Table 7. The Langmuir isotherm model provided a better correlation than the Freundlich isotherm model. It could be concluded that the sorption of Cu(II) onto IIDEMS was monolayer and the maximum monolayer sorption capacity of the sorbent was found to be 82.34 mg.g^{-1}. The adsorption capacity of IIDEMS is comparable to and moderately higher than that of many corresponding sorbents reported in the literature (Choi, *et al.*, 2003). The dimensionless separation factor, RL, was also evaluated and calculated according to Eq. (3).

$$R_L = \frac{1}{1 + K_L C_e} \qquad (3)$$

RL values can be used for the interpretation of the sorption type and it was reported that, when 0<RL<1, the sorption process is favorable (Choi, *et al.*, 2003).

Table 6: Effect of the type and concentration of the elution solutions

Type of the elution solution	Concentration (mol.L^{-1})	Recovery^{a} %
HCl	0.05	17
	0.1	99
	0.2	97
HNO_3	0.04	28
	0.1	56
	0.2	97
EDTA	0.05	98
	0.1	91
	0.2	87

(a) Mean of three replicates

Further, the RL value for this study was 1.60×10^{-3}, therefore, adsorption of Cu(II) IIDEMS was favorable.

Analysis of Cu content in real sample

The proposed SPE method possesses advantages such as easiness, and considerable selectivity in comparison with the previously reported procedures for isolation and determination of Cu(II) contents (Table 8). The maximum time taken for separation, preconcentration and monitoring of Cu(II) in 50 mL portions of water samples is at the most 10 min. The reproducibility of the procedure is near 2%. The proper preconcentration factor improves the LOD of the method by a factor of about 250. This procedure has the advantage of preconcentration of Cu(II) depending on the pH of the sample solution (Table 8).

Analytical features

Precision

The precision of the proposed method for the determination of Cu was investigated at the optimum experimental conditions (sample volume: 50 mL; pH: 5.3; flow rate: 8 Ml.min^{-1}; eluent: 10 mL 0.1 M HCl). 10

Table 7: Isotherm model parameters for the adsorption of Cu^{2+} on to IIDEMS

Langmuir			Freundlich			
q_{max} (mg.g^{-1})	K_L (L mg^{-1})	r_L^2	R_L	n	K_F (L.g^{-1})	r_F^2
74.45	0.68	0.9989	1.60×10^{-3}	6.30	29.80	0.850

Table 8: Comparison of published results of several on-line or several methods for determination of Cu(II)

Technique	Sorbent	RSD(%)	LOD (μg /L)	Ref.
FIA-AAS	Lanthanum hydroxide	-	9.5	16
FIA-AAS	1,5-Diphenylcarbazide	2.9%	20	17
ICP-AES	Titanic silicate	2.5%	49	18
Several methods	DDTC and C_{18} bonded silica disk	2.7%	7.5	Present work

independent sorption and elution cycles were carried out by following the recommended procedure. The precision of the preconcentration method was evaluated by using the relative standard deviation (RSD) and was found to be 2.7%. The mean recovery of ten replicates was 98±3% at a confidence level of 95%. The precision of the proposed method was good and the recovery of Cu was quantitative.

Calibration graph

A linear calibration curve was obtained in the concentration range of 0.21.0 μg.mL^{-1}. The calibration equation was A = 0.0025 + 0.0398C, where A is the absorbance and C is the Cu concentration in μg.mL^{-1}. Correlation coefficient was 0.9989 and the average values of triplicate readings for each standard solution were used for the calculations.

Detection limit

The value of detection limit based on three times the standard deviation of blank signal (N=20) was 7.5 ng.mL^{-1}. The detection limit of the proposed method is comparable to those obtained by other methods described in the literature (Moghimi, *et al.*, 2012; Choi, *et al.*, 2003; Kaiss, *et al.*, 2007). The corresponding limit of quantification was calculated from ten times the standard deviation of blank signal and found as 20.2 ng.mL^{-1}. As is seen, the recovered Cu ion reveals that the results are quite reliable and are in satisfactory agreement with those obtained by ICPAES.

CONCLUSIONS

A novel and selective method for the fast determination of trace amounts of Cu ions in water samples has been developed. The Cu ions was determined by using Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl] ethanethioate (IIDE) modified Octadecane-functionalized nano graphene (OD-G) sorbent. The optimum recoveries for Cu were obtained with 300 mg sorbent, at pH 5.3 and 8 mL.min^{-1} of flow rate. Cu ions were quantitatively recovered (>95%) with 10 mL of 0.1 mol.L^{-1} HCl and the preconcentration factor was 250 at optimum conditions. The modified Octadecane-functionalized nano graphene (OD-G) has high sorption capacity (82.34 mg .g^{-1}) and the equilibrium data followed by the Langmuir isotherm model. The precision of the proposed method evaluated as the relative standard deviation obtained from ten replicates, was 2.7%. In comparison to other solid phases, high flow rates and large preconcentration factor was achieved using Isopropyl 2-[(isopropoxy carbothioyl) disulfanyl] ethane thioate (IIDE) modified Octadecane-functionalized nano graphene (OD-G) sorbent. While other advantages over reported methods are the high tolerances for matrix components (Moghimi, *et al.*, 2012; Choi *et al.*, 2003; Kaiss, *et al.*, 2007) superior sorption capacity and good reusability.

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