**RESEARCH ARTICLE****SEPARATION AND EXTRACTION OF CU(II) USING NANO GRAPHENE WITH
AMINOPROPYLTRIETHOXSILANE (APTES)****ALI MOGHIMI**

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Corresponding Author: alimoghimi@iauvaramin.ac.ir**Abstract**

A simple method has been developed for the preconcentration of copper(II) based on the adsorption of its nano graphene with aminopropyltriethoxysilane (APTES). Simple chemical bonding method to synthesize nano graphene with aminopropyltriethoxysilane (APTES) was reported. A novel and selective method for the fast determination of trace amounts of Cu(II) ions in water samples has been developed. The procedure is based on the selective formation of Cu(II) ions using nano graphene with aminopropyltriethoxysilane (APTES) at different pH followed by elution with organic eluents and determination by atomic absorption spectrometry. The preconcentration factor was 100 (1 ml elution volume) for a 100 ml sample volume. The limit of detection of the proposed method is 1.0 ng ml^{-1} . The maximum sorption capacity of sorbent under optimum conditions has been found to be 5mg of Cu per gram of sorbent. The relative standard deviation under optimum conditions was 3.0% ($n = 10$). Accuracy and application of the method was estimated by using test samples of natural and synthetic water spiked with different amounts of Cu(II) ion.

Keywords: Preconcentration; Cu(II); nano graphene with aminopropyltriethoxysilane (APTES); Solid phase extraction; Flame Atomic Absorption Spectrometry (FAAS)

Introduction

Copper at trace concentrations acts as both a micronutrient and a toxicant in marine and fresh water systems [1-8]. This element is needed by plants at only very low levels and is toxic at higher levels. At these levels, copper can bind to the cell membrane and hinder the transport process through the cell wall. Copper at nearly 40 ng mL^{-1} is required for normal metabolism of many living organisms [9, 10]. On the other hand, copper 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 copper is usually carried out by flame and graphite furnace atomic absorption spectrometry (AAS) [11, 12] as well as

spectrometric methods [13, 14]. However, due to the presence of copper in medicinal and environmental samples at low levels, its separation from other elements presents and also the use of a preconcentration step prior to its determination is usually necessary.

Different methods, especially Liquid- Liquid extraction of copper in the presence of various classical [15-19] and macrocyclic [20, 21] co-extractant ligands has attracted considerable attention. However, the use of classical extraction methods for this purpose is usually time-consuming, labor-intensive and requires large amounts of high purity solvents for extraction. Nevertheless, several other techniques for the preconcentration and separation of copper have

been proposed including liquid chromatography[22] supercritical fluid extraction[23], flotation[24], aggregate film formation[25], liquid membrane[26], column adsorption of pyrocatechol violet-copper complexes on activated carbon[27], ion pairing[28], ion pairing[29], preconcentration with yeast[30], and solid phase extraction using C₁₈ cartridges and disks[31-33,46-49].

Solid phase extraction (SPE) or liquid-solid extraction is popular and growing techniques that are used to sample preparation for analysis. It is an attractive alternative for classical liquid-liquid extraction methods that reduce solvent usage and exposure, disposal costs and extraction time for sample separation and concentration purposed [34-36]. In recent years, the octadecyl-bonded silica SPE disks have been utilized for the extraction and separation of different organic compounds from environmental matrices[37-40]. Moreover, the SPE disks modified by suitable ligands are successfully used for selective extraction and concentration of metal ions [41-42]. In a recent series of papers,[43-45] we have described the application of metal-DNA conjugates to nucleic acid sequence determination with catalytic signal amplification; the assay relies on the esterase activity of a DNA-linked Cu complex. For optimization of the system and exploration of structure- activity relationships, a sensitive probe would be useful, which allows straightforward detection of esterase activity of ligated Cu²⁺ in low concentration.

In this study, we report the synthesis of this new sorbent and its application as a selective sorbent for separation, preconcentration and determination of Cu²⁺ ions by FAAS determination.

Experimental

Apparatus and Reagents

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 hollow cathode lamp(HI-HCl) according to the recommendations of the manufacturers. Separation of sorbent was assisted using a centrifuge (centurion scientific model: K 240R, West Sussex, U.K.). The pH measurements were carried out by an ATC pH meter (EDT instruments, GP 353). Infrared spectra of nano

graphene with aminopropyltriethoxysilane (APTES) were carried out from KBr by a Perkin-Elmer 1430 ratio recording spectrophotometer. Graphite oxide was prepared from purified natural graphite (SP-1, Bay Carbon, Michigan, average particle size 30 μm) by the Hummers [53]. method and dried for a week over phosphorus pentoxide in a vacuum desiccators before use. 4-Isocyanatobenzenesulfonyl azide was prepared from 4-carboxybenzenesulfonyl azide via a published procedure [54].

Synthetic procedures

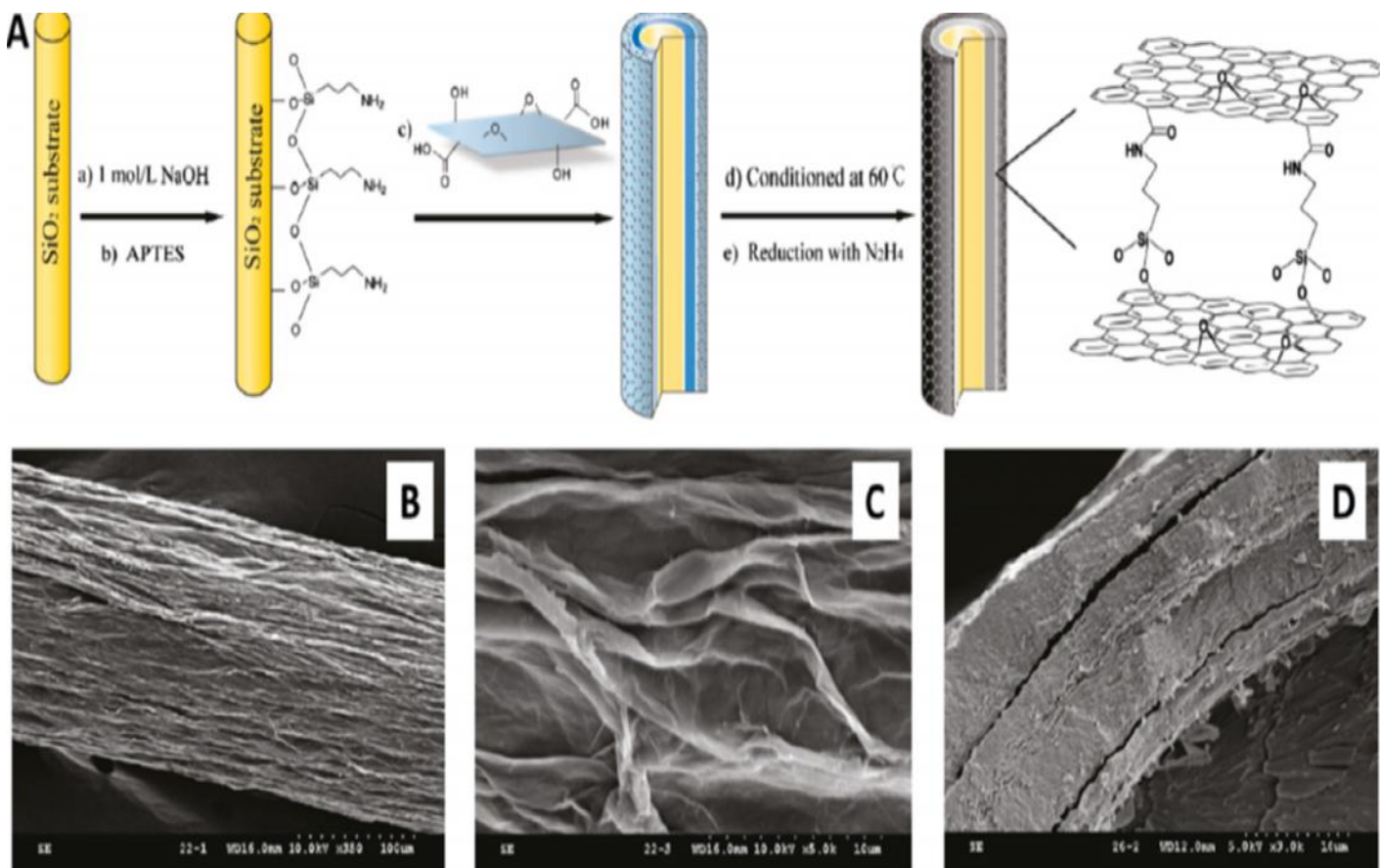
Preparation of GO-APTES

Zhang et al. reported the preparation of chemicalbonded graphene coating for SPME in a layer-by-layer manner (Fig. 1A) [50]. The silica fiber was first treated with NaOH and 3- amino propyltriethoxysilane (APTES) to introduce amino groups to the surface. Then, GO was bonded to the fiber via reaction of the carboxyl groups of GO with the amino groups. Repeating the treatment with APTES and GO four times gave a coating of 20 μm thickness (Fig. 1D). Finally, the fiber was aged at 60°C and reduced with hydrazine. SEM showed a rough tree-bark-like structure with a striped appearance (Fig. 1B), and the high-resolution image revealed a continuous folded, wrinkled structure (Fig. 1D).

Procedure

A batch-wise process was employed for the extraction and preconcentration of Cu. Extraction was performed in test tubes containing Cu²⁺ in 10 ml acetate buffered solution (pH 3.0). Sixty milligrams GO-APTES was added into the solution. After that, the mixture was shaken manually for an appropriate time to extract Cu completely from the solution. Finally, test tubes were placed in centrifuge and separation of sorbent was achieved by centrifugation for 2.5 min at 3500 rpm. The bulk aqueous phase was removed with a pipette and any residual aqueous phase was easily decanted. The back extraction was performed using 1.0 ml of 1.0 mol l⁻¹ methanol solution. The Cu concentration was determined by Flame Atomic Absorption Spectrometry (FAAS).

Figure 1. (A) Layer-by-layer fabrication processes of chemical-bonded graphene-coated SPE fiber. (B–D) SEM images of an SPME fiber coated with graphene. The surface images at magnifications of (B) 350 \times and (C) 5000 \times ; the cross-section image at magnifications of (D) 3000 \times [50].



Results and discussion

The treatment of nano graphene with aminopropyltriethoxysilane (APTES) can lead to the derivatization of both the edge carboxyl and surface hydroxyl functional groups via formation of amides [50] or carbamate esters [52], respectively.

Morphology

The amount of nano graphene with aminopropyltriethoxysilane (APTES) 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–APTES material, is attributed to the decomposition of APTES (Fig. 2).

The GO–APTES material forms a stable dispersion in DMF at a concentration not exceeding 1 mg mL⁻¹. The electronic absorption spectrum of GO–APTES 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 APTES units (the Q-bands at 516, 557, 589 and 648 nm were flattened to the base line in the GO–APTES material). Interestingly, the absorption of porphyrin in the GO–APTES material is broadened, shortened and bathochromically shifted (ca. 2 nm) as compared to that of the free APTES, 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 APTES) in the ground state. These results are in

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

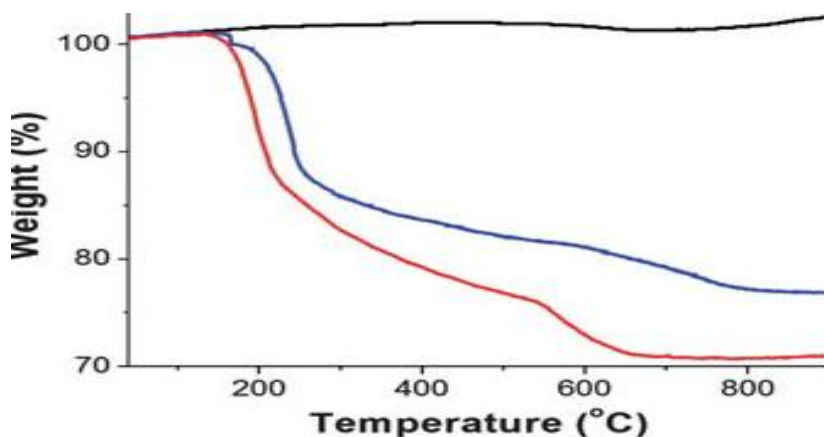
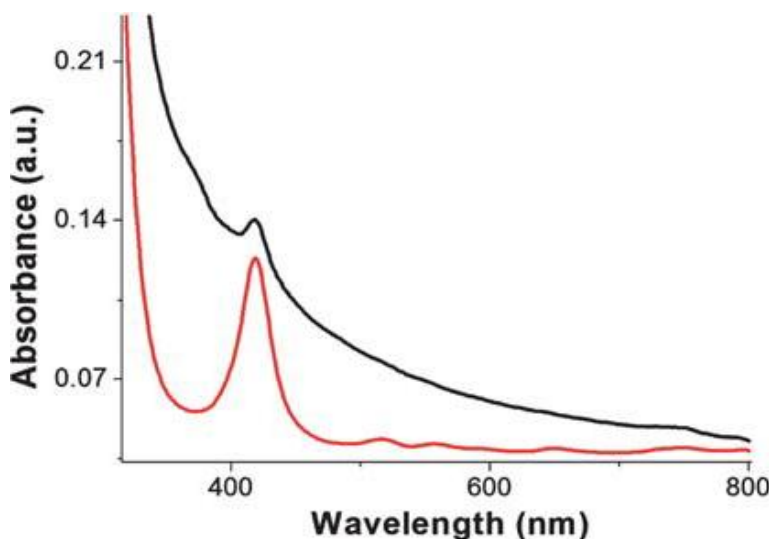


Fig. 3 The UV-vis spectra of GO–APTES (black) and free APTES (red), obtained in DMF.



agreement with studies based on other hybrid systems consisting of porphyrins covalently grafted to carbon nanotubes and nanohorns[50].

Effect of pH

Basically, the approach described above for Cu(II) detection at APS-MCPE should be applicable to any modified electrode system involving N-bearing ligands that would require a modulation of their properties by a pH change. To point out this generalisation aspect, we have revisited an earlier work dealing with the use of an amorphous silica sample functionalised with carnosine groups (Scar), which was applied as modifier of a carbon paste

electrode (Scar-MCPE) to Cu(II) preconcentration and detection[41]. The synthetic protocol to get the Scar material involved protection/deprotection processes resulting in N-bearing ligands under their protonated form, counter-balanced by chloride anions.

The effect of pH on the extraction of Cu²⁺ from water samples was studied in the pH range of 1.0–8.0. The higher pH values were not studied because functionalized GO–APTES were not stable in alkali solutions due to the breaking of the C–N–H bonds by hydroxide ions attack[54]. pH of the solution was adjusted at the

required value by adding 1.0 mol l^{-1} sodium hydroxide and/or 1.0 mol l^{-1} nitric acid. As can be seen in Fig. 4, extraction was nearly constant and quantitative in the pH range of 2–8.0. At lower pH (<2.5), the nitrogen atoms in GO–APTES are protonated, so the stability of complex formation between the sorbent and Cu^{2+} is reduced. Therefore, the extraction of Cu decreased. Hence, pH of 3.0 was chosen as the optimum pH for extraction.

Choice of eluent

In order to choose the most effective eluent for desorbing Cu ion from the sorbent surface aliquots of 10 ml of $0.1 \mu\text{gml}^{-1}$ Cu ion solution was contacted with 50.0 mg of GO–APTES. A series of selected eluent solution such as nitric acid, formic acid, acetic acid, sodium thiosulfate, ethanol and methanol was used. A total of 10.0 ml of 0.1 mol l^{-1} of the above mentioned eluents were used for desorbing the adsorbed Cu ion. The amount of Cu ion back-extracted into the liquid phase by each eluent was measured using Flame Atomic Absorption Spectrometry (FAAS). Percent recoveries of Cu ion were calculated for each sample. The results (Fig. 5) showed that recovery was the best when methanol was used as eluent. Also, higher concentrations of hydrochloric and nitric acid (0.5 and 1.0 mol l^{-1}) solutions were tested and the results showed the recovery of Cu was not quantitative. Therefore, methanol was selected as eluent.

Effect of eluent concentration

The influence of the concentration of methanol on desorption of Cu ion was studied. For desorbing $1.0 \mu\text{g}$ Cu ion, already adsorbed on 50.0 mg of sorbent, 1.0 ml of different concentration of eluent (methanol) have been used. At a concentration of more than 0.7 mol l^{-1} , methanol desorbs (recovery of almost 100%) Cu ion completely from the sorbent surface. A concentration of 1.0 mol l^{-1} of methanol was selected for further studies.

Effect of the sample volume

In order to explore the possibility of concentrating low concentrations of Cu from large volumes, the maximum applicable volume must be determined. For this purpose, the effect of the sample solution volume on the recovery was studied by keeping the total amount of Cu^{2+} uptake constant ($1.0 \mu\text{g}$).

The quantitative recoveries were obtained for sample volume of 100 ml. Therefore, the concentration factor was 100 for Cu^{2+} since the final elution volume was 1.0 ml.

Effect of the amount of GO–APTES

To test the effect of the amount of GO–APTES on quantitative retention of analyte different amounts of sorbent (range from 2.0 to 200.0 mg) were added into the solution following the experimental method. The results showed that the extraction of Cu^{2+} was quantitative by using only 10.0 mg of GO–APTES. Subsequent extraction experiments were carried out with 50.0 mg of GO–APTES in order to achieve higher capacity and to account for other extractable species.

Adsorption capacity

The capacity of the sorbent is an important factor that determines how much sorbent is required to remove a specific amount of metal ions from the solution quantitatively. For investigation of adsorption isotherm of Cu ion, the same volumes of Cu ion solution with different concentrations of Cu ion were contacted with 0.1 g of sorbent in the batch mode. Then, the concentration of the remaining Cu in the solution was determined by Flame Atomic Absorption Spectrometry (FAAS). The adsorption isotherm that is the number of microgram absorbed per gram of adsorbent (N_f) versus the equilibrium concentration of cation (C_s) is shown in Fig. 6. According to these results, the maximum amount of Cu that can be sorbed by GO–APTES was found to be 5 mg g^{-1} at pH 3.0.

Effect of equilibrium time

In order to investigate the effect of shaking time on the extraction efficiency, extraction for a series of solutions containing $1.0 \mu\text{gCu}^{2+}$ were carried out. The results showed that the shaking time (from 20 to 350 s) has no effect on the extraction efficiency of Cu and the extraction was quantitative. Although the extraction process can be continued during the centrifugation, however, the results showed that extraction was quantitative and very fast in all cases. Thus, the mixtures have been shaken for 20 s to reach equilibrium in the subsequent experiments.

Effect of ionic strength

The influence of ionic strength on the extraction of Cu was studied in the potassium nitrate solution with various concentrations from 0.01 to 1.0 mol l⁻¹. Results have shown that ionic strength has no considerable effect upon extraction efficiency up to 1.0 mol l⁻¹ of KNO₃. These observations showed the specific tendency of GO-APTES for Cu²⁺ and the possibility of using this method for separation of Cu from highly saline solutions.

Effect of coexisting ions

The effects of common coexisting ions in natural water samples on the recovery of Cu were studied. In these experiments, 10 ml of solutions containing 0.1 µg l⁻¹ of Cu and various amounts of interfering ions were treated according to the recommended procedure. An ion was considered to interfere when its presence produced a variation in the extraction recovery of sample more than ±5%. The results showed that, in excess of 10,000-fold Li⁺, K⁺, Na⁺, Ca²⁺, Mg²⁺, Ba²⁺, Sr²⁺ and 1000-fold Cl⁻, Br⁻, SO₄²⁻, Ag⁺, Co²⁺, Cu²⁺, Ni²⁺, Zn²⁺, Mn²⁺, Pb²⁺, Al³⁺, Cr³⁺, Fe³⁺ and Hg²⁺ ions had no significant interferences in the extraction and determination of Cu. As can be seen, GO-APTES has shown a high tolerance limit for alkali and alkaline earth metals. This is particularly useful for the analysis of Cu in natural water samples, for example, seawater, which contain large amounts of alkali and alkaline earth metal ions.

Reusability and stability of GO-APTES

Reusability is one of the key parameters to assess the effectiveness of a sorbent. A series of sorption/desorption experiments were performed to understand the reusability of the synthesized GO-APTES. After sorption, the sorbent was treated with 1.0 mol l⁻¹ methanol to desorb Cu²⁺ and this sorption/desorption procedure was repeated five times. After each desorption step, the sorbent was washed with doubly distilled water to remove methanol and condition sorbent. On storing for a year under dark and dry conditions, the stability of sorbent was excellent and adsorption capacity did not change significantly.

Analytical Performance

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as the

amount of analyte necessary to yield a signal equal to three times (3) and ten times (10) the standard deviation of the blank signals, respectively. Using sample volume of 100 ml a LOD of 1.0 µg l⁻¹ and a LOQ of 1.2 µg l⁻¹ were obtained for the determination of Cu. Ten replicate extraction and measurement of 1.0 µg of Cu²⁺ ion in 100 ml water solution gave a R.S.D. of 3.0%. Calibration graph was obtained using preconcentration of 100 ml of standard solutions buffered at pH 3.0 with 50 mg of sorbent. For this purpose, standard solutions containing Cu ion in the range of 1–1000 µg l⁻¹ were examined by the proposed procedure and it was observed that calibration curve were linear in this range. The regression equation was $I = 0.0098C (\mu\text{g l}^{-1}) + 0.0049$ and the correlation coefficient was 0.9998.

Analysis of water samples

To assess the applicability of the method to real samples, it was applied to the extraction and determination of Cu from 100 ml of different water samples. Tap water (Tehran, taken after 10 min operation of the tap), rain water (Tehran, 20 January, 2014), and Sea water (taken from Caspian sea, near the Mahmoud-Abad shore) samples were analyzed (Table 1). As can be seen from Table 1 the added Cu ions can be quantitatively recovered from the water samples used.

Conclusion

Results presented in this work demonstrate well the tremendous possibilities offered by the solid phase extraction of trace amounts of Cu(II) in water samples using of GO-APTES and its determination by FAAS. The method developed was simple, reliable, high capacity, good stability and fast adsorption and desorption kinetics for determining Cu in water. Also, the proposed method was free of interference compared to conventional procedures to determine Cu [48-51]. The method can be successfully applied to the separation and determination of Cu in binary mixtures.

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