Comparative Methods for Online Preconcentration of Copper Ions on Epichlorohydrin Cross Linked Silica Gel-chitosan Composite Beads and Epichlorohydrin Crosslinked Multi Walled Carbon Nanotube-Chitosan Composite Beads by Flow Injection System

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In this work, two different adsorbents were comparatively used and developed for preconcentration of ultra-trace levels of copper ions by flow injection analysis for atomic absorption spectrometry. Copper ions were preconcentrated on a mini-column packed with epichlorohydrin cross-linked silica gel-chitosan composite beads and epichlorohydrin cross linked multiwalled carbon nanotube-chitosan composite beads. 0.1 M ethylene diamine tetra acetic acid and 0.1 M nitric acid solutions were used in order to remove the copper ions from the adsorbents. The factors affecting preconcentration of copper(II) ions such as sample and eluent flow rate, loading time, pH, eluent concentration, and interfering ions were investigated. Under the optimized parameters, detection limits of the methods were obtained as 2 ng ml^{-1} for epichlorohydrin cross linked silica gel-chitosan composite beads and 1.3 ng ml^{-1} for epichlorohydrin cross linked multiwalled carbon nanotube-chitosan composite beads with enrichment factor of 23 and 19.2, respectively. The recoveries and intra-day and inter-day precisions for copper ions were >90.0% and >3%, respectively. In order to validate the developed methods, a certified reference material of drinking water was analyzed. The values determined were in good agreement with the certified values. The developed methods have also been applied for determination of copper(II) ions in Gediz River in Turkey with satisfactory results.

Keywords: Copper, Flow injection analyses, Silica gel-chitosan composite beads, Carbon nanotube-chitosan beads, Online preconcentration

INTRODUCTION

Electric industries, smelting and refining galvanization, fertilizers, and mining activities are the potential sources of heavy metal ions in waste waters. A major environmental problem is toxic heavy metal contaminations of the environment. So, development of heavy metal determination methods is very important. Many methods have been developed for copper determination, such as atomic absorption, inductively coupled plasma emission spectrophotometry, and inductively coupled plasma mass spectrometry. In recent years, flow injection online preconcentration has shown great promise and become one

of the most active research fields in automated solution [1].

Flow injection analyses (FIA) allow inexpensive automation of chemical analysis. Moreover, it works in a closed system with a significant reduction of airborne contamination and a fairly high sampling frequency, low detection limits and high repeatability [2]. As in FIA all conditions are reproduced, dispersion is very controlled and reproducible. That is why, all samples are sequentially processed in exactly the same way during passage through the analytical channel, in other words, what happens to one sample exactly happens for other samples. FIA is a microchemical technique which consumes minute quantities of sample and reagents, and therefore, generates less than 1 ml of waste per assay. Preconcentration techniques are usually required in order to determine such low levels of

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metals. However, the direct determination of trace amounts of heavy metals in complex matrixes is usually difficult due to matrix interferences or insufficient detection power. In order to overcome these problems, separation and preconcentration of the given analytes become indispensable, particularly when they exist at trace levels of concentration. Accurate analytical determination of metal ions at trace levels requires versatility, specificity, sensitivity, and accuracy. Several online preconcentration methods have been reported. These include liquid-liquid [3] and liquid-solid [4] extraction processes performed in the presence of various complex agents. This is usually carried out with flow injection analyses of copper using a mini-column. Preconcentration technique has several advantages over the other techniques, such as; it is rapid, reproducible, and requires only small volumes of solvents [5,6]. A great variety of functionalized materials have been used as sorbents for online preconcentration systems for the determination of several metals. Polymeric chelating resins [7], silica gel grafted with adsorbents [8, 9], nanoporous materials [10,11] and ion imprinted polymers [12], are examples of these materials. In recent years, the use of modified silica gels in the preconcentration of trace metals has been investigated. Silica gel is the most widely used polar adsorbent, especially in its deactivated form in solid phase applications. Main advantages over other solid supports are relative activity, large adsorption capacity, easy preparation of different types with different pore size and total surface area under standard conditions. In addition, the possibility of surface modification or coating with a reagent is also reported as an advantage. Modified silica gels offer the advantages of high surface areas and increased mechanical stability. In recent years, both single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs) have attracted much interest in exploiting unique thermal, electronic, mechanical, physical and chemical properties. The extremely large surface area, the unique tubular structure, and the hexagonal arrays of carbon atoms have made CNTs a promising adsorbent material. The highly developed hydrophobic surface of CNTs exhibits strong sorption properties toward various organic compounds and metal ions [12].Of the biopolymers, chitosan has been receiving special attention because it is derived from chitin, which is the second most abundant

biopolymer in nature and a waste product of seafood processing industries [13]. Furthermore, chitosan has biological and chemical properties such as high chemical reactivity, biocompatibility, non-toxicity, anti-bacterial properties, chirality, chelation and adsorption properties [14-20]. Chitosan is well established as an excellent natural adsorbent because amine and hydroxyl groups may serve as coordination sites to form complexes with various heavy metal ions [21]. Chitosan is soluble in most dilute mineral acids. Consequently, the chemical stability needs to be reinforced through treatments using crosslinking agents for application in acidic media. The crosslinking procedure may be performed by reaction of chitosan with different agents such as glutaraldehyde, ethylene glycol diglycidyl ether and epichlorohydrin [22-24]. Epichlorohydrin is a crosslinking mono-functional agent used to form covalent bonds with the carbon atoms of the hydroxyl groups of chitosan, resulting in the rupturing of the epoxide ring and the removal of a chlorine atom [25]. Chitosan and its derivatives are capable of adsorbing a number of metal ions as its amino groups can serve as chelation sites. Due to their high nitrogen content and porosity, silica gel and carbon nanotubes chitosan have exhibited relatively high sorption capacities for heavy metals.

In this work, simple, efficient, selective, cost-effective, rapid and reproducible online procedure is proposed for preconcentration and determination of copper at low concentration levels by FIAAS using a mini-column packed with silica gel-chitosan composite beads and carbon nanotube-chitosan composite beads. Experimental conditions were used to optimize the operational parameters of the FIFAAS systems. Analysis of certified reference materials of EnviroMAT Drinking Water, Low (EP-L-2) showed good agreement with the certified value. This procedure was applied to the determination of Cu(II) in river water samples from Gediz River.

MATERIALS AND METHODS

Apparatus

Measurements were carried out by Varian 220 FS model atomic absorption spectrometry. All solutions were pumped with Tygon tubes by the Heidolph peristaltic pump with four channels. Rheodyne six-way rotary injection valve was

used in the flow injection system. Hanna P211 microprocessor pH-meter using a combined glass electrode was used to measure the pH of the solutions. Philips (FEI) XL30-SFEG scanning electron microscope was used to investigate the surface morphology of the samples.

REAGENTS AND SOLUTION

All the reagents used were of analytical reagent grade (Sigma St. Louis, MO, USA and Merck). Ultrapure de-ionized water used in all the study was purified by Milipore ultrapure water purification system. All laboratory glassware were kept in a 10% (v/v) nitric acid solution overnight and then rinsed with deionized water. The working standard solutions of copper(II) were prepared using appropriate dilutions of stock solutions (1000 mg l^{-1}) obtained from Merck. Buffer solutions were used at a concentration of 0.1 M to adjust the pH: citric acid/sodium citrate buffer (pH 3.0), acetic acid/sodium acetate (pH 4.0-6.0), sodium dihydrogen phosphate/disodium hydrogen phosphate (pH 6.0-8.0), and ammonium buffer solutions (pH 9-10). Ethylene diamine tetra acetic acid (from Merck), hydrochloric acid and nitric acid (from Merck) were prepared and used as eluting solutions.

Flow Injection System Descriptions

The flow injection system diagram is shown in Fig. 1. Sample load and eluting position are seen in Fig. 2. Four-line peristaltic pump was used in the system. The system consists of a six-way eluent injection valve and mini column packed with a silica gel-chitosan and carbon nanotube-chitosan composite beads. The connection tubing was 1.5 mm i.d. polytetrafloroethylene and mini column was 5 cm × 3 mm i.d. polytetrafloroethylene.

Activation of Multiwalled Carbon Nanotubes

Multiwalled carbon nanotubes (MWCNTs) were activated by refluxing with 3:1 (v/v) concentrated H_2SO_4-HNO_3 acid mixture at 55 °C for 3.5 h in a round bottom flask. Multiwalled carbon nanotube-acid suspension was then centrifuged at 10000 rpm for 30 min. After centrifugation, multiwalled carbon nanotubes were repeatedly washed with deionized water until it became neutral.

Preparation of Epichlorohydrin Cross Linked Silica Gel-chitosan and Carbon Nanotube-chitosan Composite Beads

Chitosan powder was dissolved in acetic acid (2% v/v) solution [26]. Then, 20 g silica gel for silica gel-chitosan beads and 150 mg multiwalled carbon nanotube for carbon nanotube-chitosan beads with 30 ml distilled water was added. The solution was kept overnight. After this time silica gel-chitosan and carbon nanotube-chitosan suspension solutions were dropped into tripolyphosphate (pH was adjusted to 8.2 by 0.1 N hydrochloric acid solution) using syringe needle. Beads were stirred for 4 h for hardening and then the beads were filtered and washed with distilled water. Silica gel-chitosan and carbon nanotube- chitosan composite beads were dried at 40 °C for 12 h, and stored in a desiccator. 50 ml of 2.0 M sodium hydroxide, 0.266 g of sodium borohydride and 5 ml of epichlorohydrin were added to silica gel-chitosan and carbon nanotube-chitosan composite beads in an Erlenmeyer and then the beads were incubated in 40 mM 250 ml epichlorohydrin at 37 °C for 15 h for crosslinking. After the reaction, the epichlorohydrin cross linked silica gel-chitosan and carbon nanotube-chitosan composite beads were filtered and rinsed with distilled water and dried at 50 °C in oven. The epichlorohydrin cross linked silica gel-chitosan and epichlorohydrin cross linked carbon nanotube-chitosan composite beads particle size were 100-150 μm.

Preparation of Epichlorohydrin Cross Linked Silica Gel-chitosan and Carbon Nanotube-chitosan Composite Beads Column

In this study, epichlorohydrin cross linked silica gel-chitosan composite beads and epichlorohydrin cross linked carbon nanotube-chitosan composite beads were used. 0.050 g epichlorohydrin cross linked silica gel-chitosan composite beads or carbon nanotube-chitosan composite beads were filled into a mini-column between two glass wool plugs. Although polytetrafloroethylene tubing is 5 cm × 3 mm i.d., epichlorohydrin cross linked silica gel-chitosan or carbon nanotube-chitosan composite beads were packed in 1 cm.
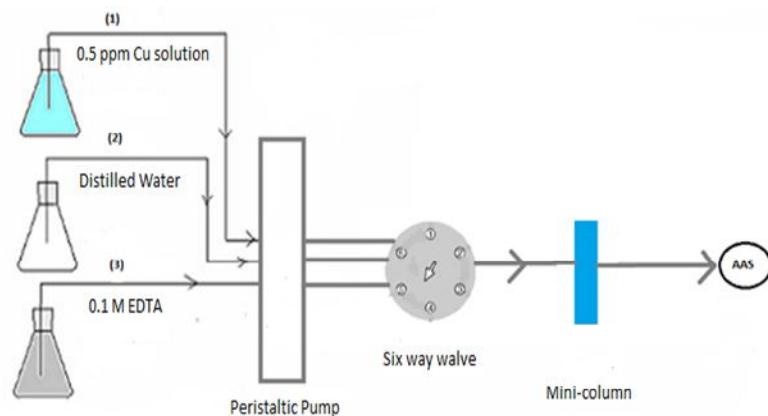
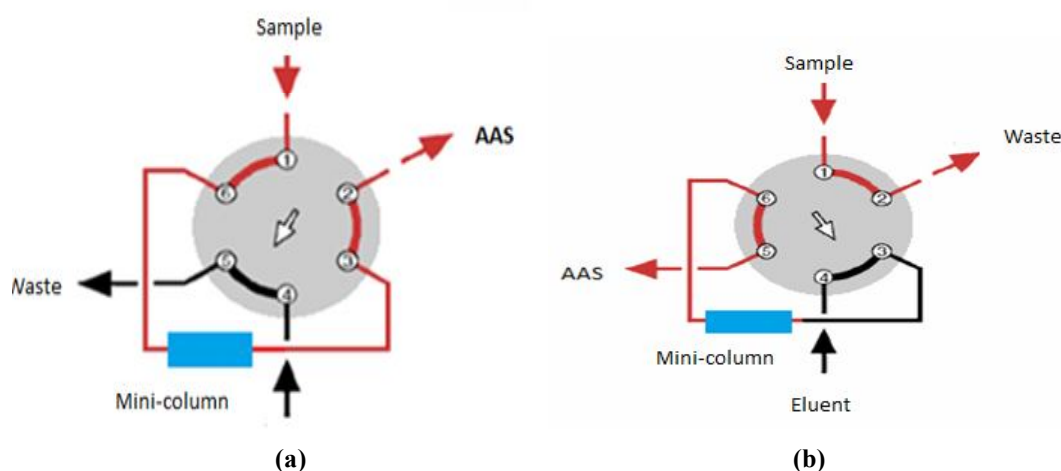**Fig. 1.** Scheme of online preconcentration flow injection system.

(a)   **(b)**

Fig. 2. a) Sample load position. b) Eluting position.

Characterization of Epichlorohydrin Cross Linked Silica Gel-chitosan and Carbon Nanotube-chitosan Composite Beads

Surface morphology of the beads was determined by scanning electron microscope (SEM) (XL30-SFEG, FEI/Philips). The weight loss temperatures of the epichlorohydrin cross linked silica gel-chitosan and carbon nanotube-chitosan composite beads were determined with a Perkin-Elmer Diamond TA/TGA (thermo gravimetric analyses) in the temperature range of 30-1000 °C at a heating rate of 10 °C per minute.

Preconcentration Methods

The preconcentration processes consists of three steps the preconcentration, washing and the elution steps. Preconcentration step: 0.5 µg ml^{-1} of copper solution or sample solution was continuously pumped into the system (Fig. 2a) in 2 min. Washing step: distilled water was pumped into the column for washing for 30 s. Elution step starts after 2.5 min of preconcentration. The valve was switched to third position so that the eluent was pumped into the column (Fig. 2b). The absorbance signal was registered and the valves were again switched to the initial position in order to have the system ready for the next

Table 1. Operating Conditions for the On-line Preconcentration of Copper on Epichlorohydrin Crosslinked Chitosan-silicagel Composite Beads and Epichlorohydrin Cross-linked Carbon Nanotube-chitosan Composite Beads

Parameter	Conditions
Wavelength (nm)	324.8
Lamp current (mA)	4.0
Slit width (nm)	0.5
Air flow rate	13.50
Acetilen flow rate (l min^{-1})	2.0
Sample flow rate (ml min^{-1})	2.0
Eluent flow rate	2.0
Time of elution (s)	20.0
Sorbent amount (g)	0.5
Minicolumn column size (cm)	5.0

preconcentration step. The samples were filtered for purification by blue band filter paper.

RESULTS AND DISCUSSION

Optimization of Operating Conditions

Preconcentration and determination operating conditions of copper are shown in Table 1. The optimized conditions were selected considering best reproducibility and maximum signal using a copper(II) standard of 0.5 µg ml^{-1} for epichlorohydrin cross linked silica gel-chitosan beads and 0.2 µg ml^{-1} for carbon nanotube-chitosan composite beads in all cases.

Effect of Sample and Eluent Flow Rate

The sample and eluent flow rate are important factors affecting the recovery of the studied metal ions on the mini-column containing epichlorohydrin cross linked silica gel-chitosan and carbon nanotube-chitosan composite beads. The effect of sample and eluent flow rate on copper(II) preconcentration was considered by varying the flow rate

(60-120 rpm) from 0.8 to 1.6 ml min^{-1}. Recovery increased with increasing flow rate, however, as seen in Fig. 3, the flow rate is reduced by 1.5 ml min^{-1} (110 rpm) for silica gel-chitosan and the flow rate is reduced by 1.35 ml min^{-1} (90 rpm) for carbon nanotube-chitosan composite beads. Therefore, 1.5 ml min^{-1} (110 rpm) was selected as eluent and sample flow rate for silica gel-chitosan, and 1.35 ml min^{-1} (90 rpm) was selected as eluent and sample flow rate for carbon nanotube-chitosan composite beads.

Effect of Sample Loading Time

Figure 4 shows a relative increase of recovery when the flow rate increased from 0.5 to 4.5 min. Maximum recovery was observed at 3.5 min. The time of the analyte loaded into the column was measured 2 min. Therefore, the optimized loading time in the present work was 1.5 min for silica gel-chitosan composite beads, and 2.0 min was selected as loading time for carbon nanotube-chitosan composite beads.

Effect of Eluent Type

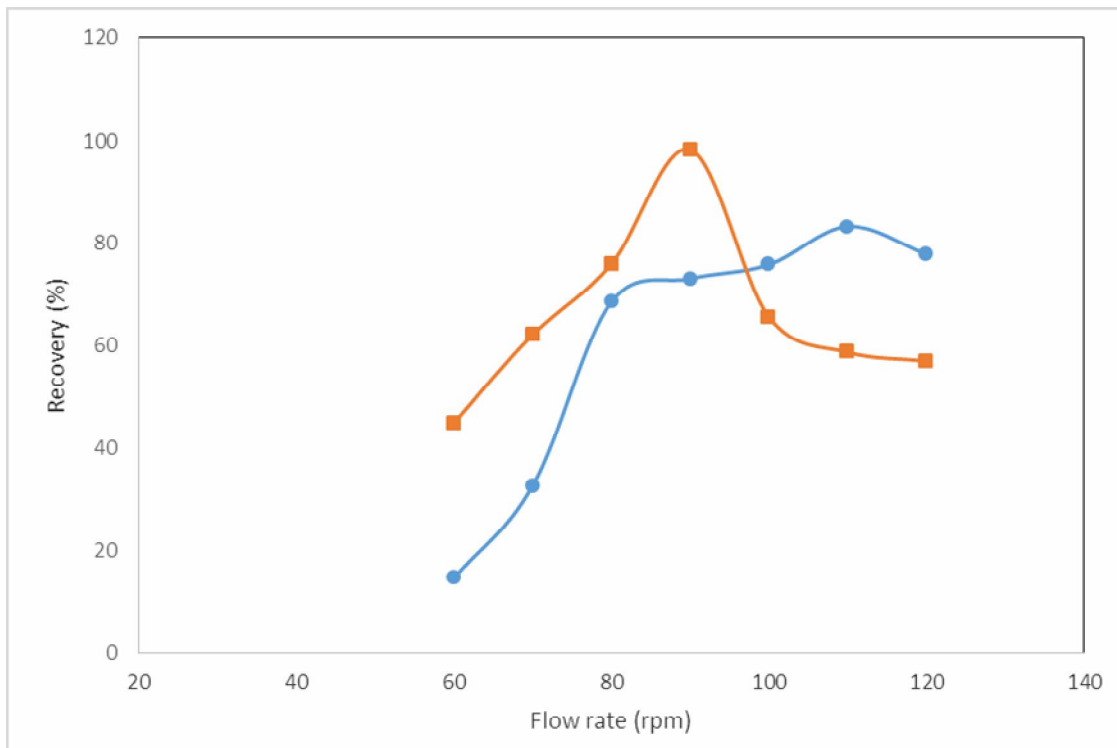Different concentrations of ethylene diamine tetra acetic

Fig. 3. Effect of flow rate (0.5 µg ml^{-1} sample concentration, eluent: 0.1 M EDTA) (● silica gel-chitosan beads, ■ carbon nanotube-chitosan beads).

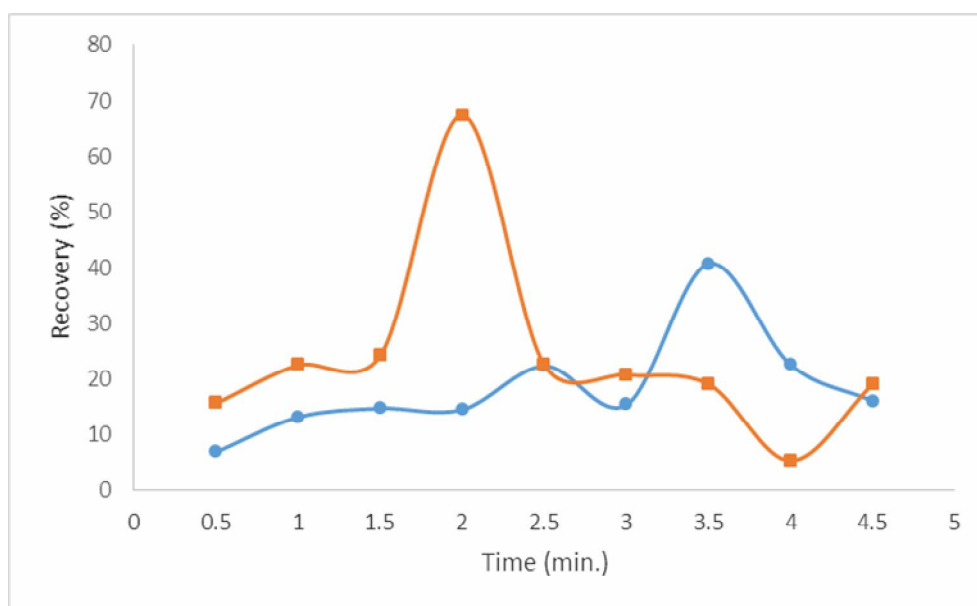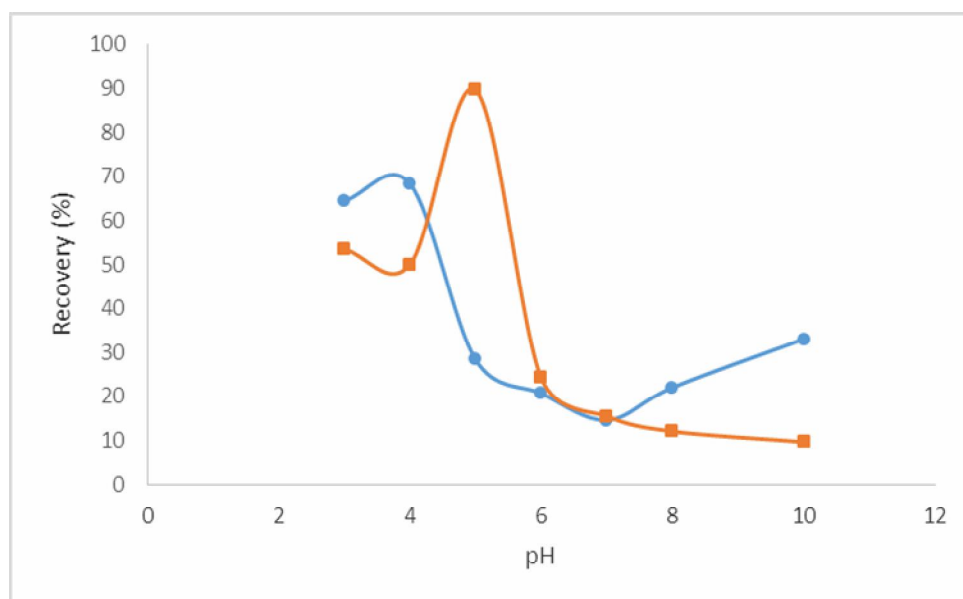**Fig. 4.** Effect of copper loading time (0.5 µg ml^{-1} sample concentration, eluent: 0.1 M EDTA, sample and eluent flow rate: 1.5 ml min^{-1}) (● silica gel-chitosan beads, ■ carbon nanotube-chitosan beads).

Table 2. Effect of the Eluent Type (0.5 µg ml^{-1} Sample Concentration, Sample and Eluent Flow Rate: 1.5 ml min^{-1}, Sample Loading Time: 1.5 min

	Recovery, standard deviation (%R) ± SD n = 5	
Eluent type	Silica gel-chitosan beads	Carbon nanotube-chitosan beads
Ethylene diamine tetra acetic acid (0.1 M)	99 ± 1.0	39 ± 2.0
Hydrochloric acid (0.1 M)	13 ± 2.0	94 ± 1.0
Nitric acid (0.1 M)	51 ± 2.0	100 ± 1.0

Fig. 5. Effect of pH. (0.5 µg ml^{-1} sample concentration, sample loading time: 1.5 min, sample and eluent flow rate: 1.5 ml min^{-1}, Elution: 0.1 M EDTA) (● silica gel-chitosan beads, ■ carbon nanotube-chitosan beads).

acid, hydrochloric acid and nitric acid solutions were used as an eluent for copper(II) in flow injection online preconcentration system. It was found that 0.1 M ethylene diamine tetra acetic acid was optimal for the elution of copper(II) from the epichlorohydrin cross linked silica gel-chitosan composite beads and 0.1 M nitric acid was optimal for the elution of copper(II) from the epichlorohydrin cross linked multiwalled carbon nanotube-chitosan composite

beads at the experimental conditions. Therefore, they were used in the following experiments as eluent (Table 2).

Effect of Sample pH

Sample pH is a significant parameter in flow injection online systems for metal ions. As seen in Fig. 5, the recovery of copper(II) ions of solution increases with the increase of pH until a maximum limit and then recovery

decreases while pH increases. Maximum recovery was obtained at pH 4.0 for epichlorohydrin cross linked silica gel-chitosan composite beads. As seen in Fig. 5, maximum recovery was obtained at pH 5.0 for carbon nanotube-chitosan composite beads. Therefore, optimum pH was selected pH 4.0 for epichlorohydrin cross linked silica gel-chitosan composite beads, and it was selected 5.0 for carbon nanotube-chitosan composite beads.

Effect of Interference Ions

Interference ions are important problems in the determination of the analytes in real samples. Interferences of various cations on the preconcentration and determination of copper was examined by comparing the signal intensities of copper(II) ions in the presence and absence of interfering ions. For this purpose, 0.5 µg ml^{-1} copper(II) with and without interfering ions (50 mg l^{-1}) were passed at 1.5 ml min^{-1} rate for 1.5 min through the epichlorohydrin cross linked chitosan-silica gel composite beads columns, and then the collected copper(II) was eluted by injecting 0.1 M ethylene diamine tetra acetic acid. The results show that in the presence of lead(II) (Pb^{2+}), sodium (Na$^+$), potassium (K$^+$), and nickel (Ni^{2+}) ions recovery was affected by approximately 30% for epichlorohydrin cross linked chitosan-silica gel composite beads column. 0.2 µg ml^{-1} copper (II) with and without interfering ions (50 mg l^{-1}) were passed at 1.35 ml min^{-1} rate for 2.0 min through the epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads column, and then the collected copper(II) was eluted by injecting 0.1 M nitric acid. The effects of the matrix ions are shown in Table 3. The results show that in the presence of nickel(II) ions recovery was affected by approximately 20% for epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads column. Interferences were also performed for anions such as nitrate, sulphate and chloride. The interference effects of these ions were found to be less than 2-3%.

Adsorption Capacities for Copper(II)

0.5 µg ml^{-1} copper as copper(II) solution was allowed to pass through a column containing 0.0502 g epichlorohydrin cross linked silica gel-chitosan composite beads for copper(II) adsorption at a flow rate of 1.5 ml min^{-1}. Copper

was measured in every 1.5 ml portion of the eluate. No copper was detected in the effluents until 15.0 ml of the copper(II) solution as shown in Fig. 6. The adsorption capacity was calculated as 0.02 mg g^{-1} copper(II) for epichlorohydrin cross linked silica gel-chitosan composite beads. 0.2 µg ml^{-1} copper as copper(II) solution was allowed to pass through a column containing 0.0502 g epichlorohydrin cross-linked multiwalled carbon nanotube - chitosan composite beads for copper(II) adsorption at a flow rate of 1.35 ml min^{-1}. Copper was measured in every 1.5 ml portion of the eluate. No copper was detected in the effluents until 15.0 ml of the copper(II) solution, as shown in Fig. 6. The adsorption capacity was calculated as 0.01 mg g^{-1} copper(II).

Analytical Features

Under the optimized conditions, the calibration graph obtained using flow injection analyses system by collecting copper(II) in the epichlorohydrin cross-linked silica gel-chitosan composite beads column was linear and had high correlation coefficient in the range studied, 0.005-1.0 µg ml^{-1} (Fig. 7). The least square equation at above dynamic linear range was as follows: y = 0.0893x + 0.002 (R^2 = 0.9970) for copper(II). The relative standard deviation was found 4% for ten replicate separate column measurements. The limits of detection, defined as LOD = 3Sb/m, where Sb is the standard deviation of 10 replicate blank signals and m is the slope of the calibration curve. Under the optimum conditions after preconcentration, the LOD for copper(II) was calculated to be 0.0020 µg ml^{-1}. Limit of quantification (LOQ): Lowest amount of analyte in a sample which can be quantitatively determined with a suitable precision and accuracy. Limit of quantification (LOQ) after preconcentration was found to be 0.0066 µg ml^{-1}. The enrichment factor (EF) was calculated as the ratios between the slopes of the linear calibration curves with and without the online preconcentration step. EF value of copper(II) was determined as 23 for epichlorohydrin cross-linked silica gel-chitosan composite beads column. Calibration graphs of copper(II) preconcentration on the epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads column was linear, and had high correlation coefficient in the range studied, 0.005-0.25 µg ml^{-1} (Fig. 7). The equations were y = 0.0294x + 0.0014 (R^2 = 0.9959) for

Table 3. Effect of Matrix Ions

Forein ion	Concentration (mg l^{-1})	Recovery, standard deviation (%R) ± SD n = 5	Recovery, standart deviation (%R) ± SD n = 5
		Silica gel-chitosan beads	Carbonnanotube-chitosan beads
Potassium	50.0	72 ± 2.0	92 ± 1.0
Sodium	50.0	70 ± 2.0	79 ± 2.0
Calcium	50.0	94 ± 1.0	100 ±1.0
Nickel	50.0	74 ± 1.0	71 ± 2.0
Zinc	50.0	86 ± 2.0	90 ± 1.0
Lead	50.0	70 ± 1.0	87 ± 1.0

Fig. 6. Breakthrough curve of the mini column for Cu(II) (0.5 µg ml^{-1} sample concentration, sample loading time:1.5 min., sample flow rate: 1.5 ml min^{-1}, ECH crosslinked chitosan-silica gel beads: 0.0502 g for Cu(II)) (● silica gel-chitosan beads, ■ carbon nanotube-chitosan beads).

copper(II). The relative standard deviation was found to be 4.1% for ten replicate measurements. Under the optimum conditions, the LOD for copper(II) was calculated to be 0.0013 µg ml^{-1}. LOQ was found to be 0.0084 µg ml^{-1}. EF value of copper(II) was determined as 19.2 for epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads column. Table 4 shows general performance parameters for reported literatures for
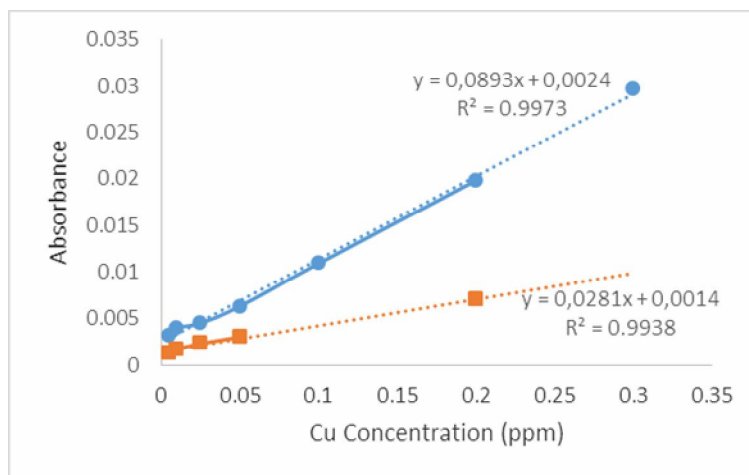
Fig. 7. Calibration graph for the determination of Cu(II) (pH = 4.0, Flow rate: 1.5 ml min^{-1}, Load time: 1.5 min, Elution: 0.1 M EDTA (● silica gel-chitosan beads, ■ carbon nanotube-chitosan beads).

Table 4. General Performance Parameters for Reported Literatures for Copper Analysis

Adsorbent	Working range	Limit of detection	Preconcentration factor	Ref.
Nano-TiO$_2$ modified with 2-mercaptobenzothiazole	0.2-20.0 ng ml^{-1}	0.15 ng ml^{-1}	234	(Pourreza *et al*., 2014) [29]
dithiooxamide, into microcrystalline cellulose	0.058 and 0.072 mmol g^{-1}		50	(Jorgettoa *et al*., 2013) [30]
2,9-Dimethyl-4,7-dipenthyl-1,10-phenanthroline	0.28-3.08 µg l^{-1}	0.80 µg l^{-1}	25	(Mendil *et al*., 2015) [31]
4-(2-Thiazolylazo) resorcinol-modified activated carbon	-	0.19 µg l^{-1}	-	(Habila, *et al*., 2014) [32]
Carbon nanotubes	-	0.5 ng ml^{-1}	-	(Zawiszan *et al*., 2012) [33]
Silica gel-chitosan carbon nanotubes-chitosan beads	0.005-1.0 µg ml^{-1}	0.0020 µg ml^{-1} 0.0013 µg ml^{-1}	23 19.2	This work

copper(II) ion analysis Accuracy studies: Certified reference material and real sample analysis. Certified reference material (EnviroMAT Drinking Water, Low (EP-L-2)) was used for the validation of the proposed method. As Table

5 shows, a good correlation was obtained between the reported amounts and the amounts found by the current method. Therefore, this method can be used as a reliable technique for determination of copper in environmental

samples. The method for the determination of copper(II) was applied to the samples of the Gediz River in Manisa. The results were obtained 50 µg ml^{-1} for copper(II), as shown in Table 5.

Characterization of Adsorbents

Scanning electron microscope pictures of epichlorohydrin cross linked chitosan-silica gel composite beads are shown in Fig. 8a, the copper-sorbed

Table 5. Analysis of Standard Reference Material, (EnivroMat EP-L-2 CRM) and Natural Water Samples

	Analyte	Certified value (µg ml^{-1})	Found (µg ml^{-1}) (n = 5)	Recovery (%)	RSD (%)
Silicagel-chitosan	CRM	0.0160 ± 0.002	0.0157 ± 0.004	98.125	4.0
Carbonnanotube-chitosan beads	CRM	0.0160 ± 0.002	0.0170 ± 0.004	106.25	4.0
Silicagel-chitosan	The Gediz river water	-	50.00 ± 0.004		
Carbonnanotube-chitosan beads	The Gediz river water	-	55.0 ± 0.004		

CRM: Certificated reference material.

Fig. 8. a) SEM pictures of ECH crosslinked chitosan-silicagel composite beads. b) After Cu(II) sorbed. c) After Cu(II) desorbed ECH crosslinked chitosan-silicagel composite beads by EDTA.
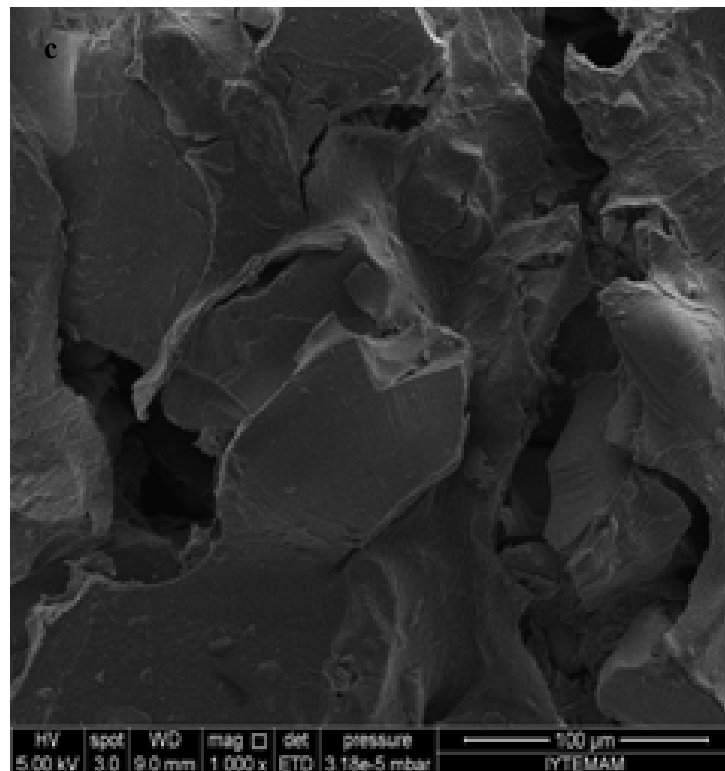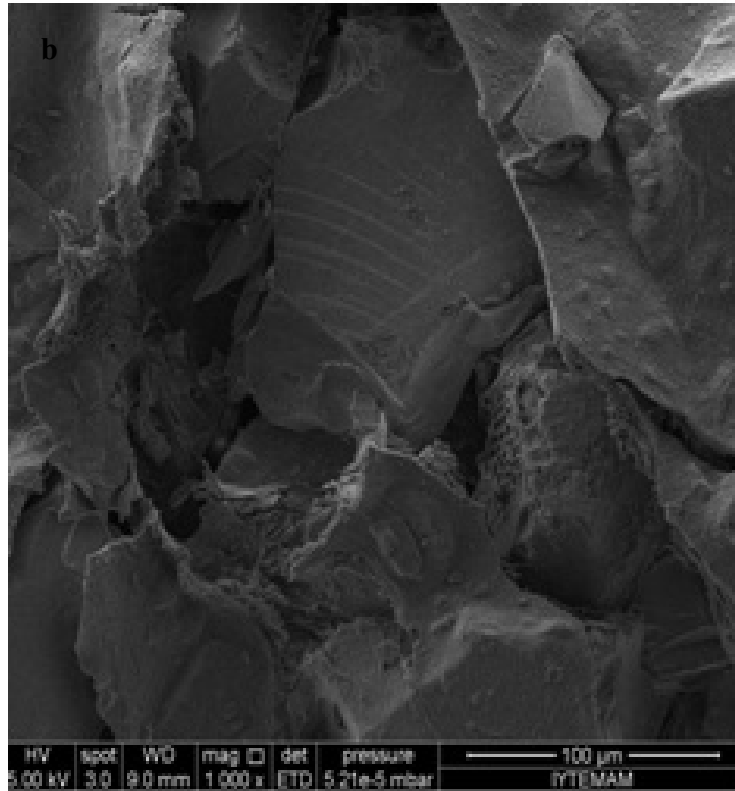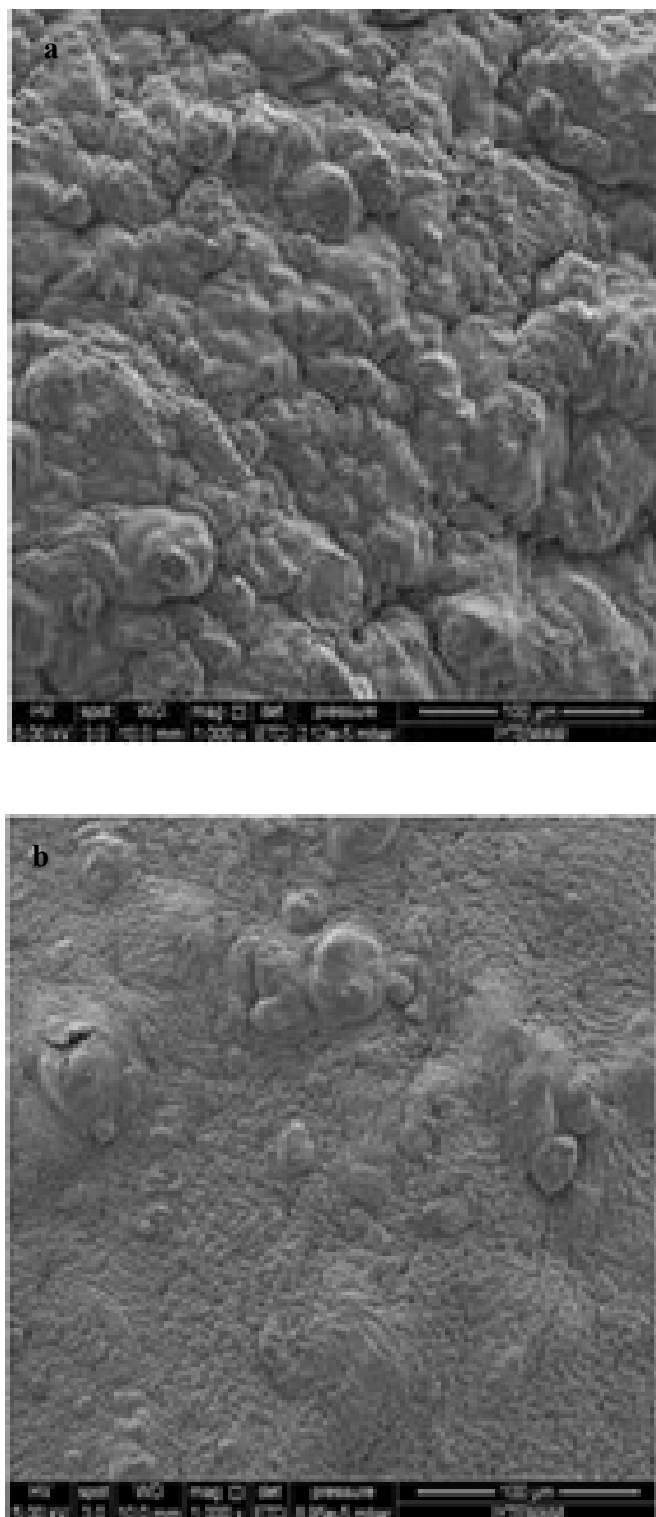
Fig. 8. Continued.

Fig 9. a) SEM pictures of ECH cross-linked multiwalled carbon nanotube-chitosan composite beads b) after Cu(II) sorbed c) after Cu(II) desorbed ECH cross-linked multiwalled carbon nanotube-chitosan composite beads.
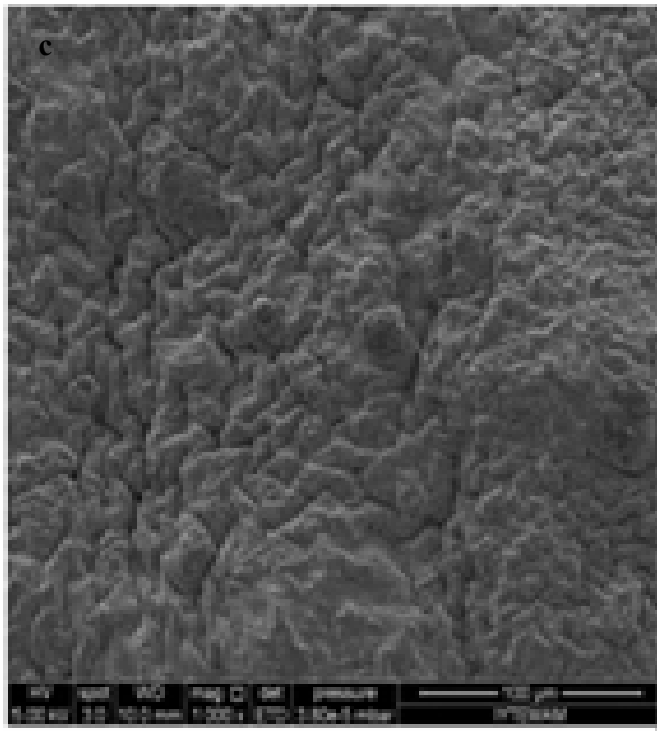
Fig 9. Continued.

epichlorohydrin cross linked chitosan-silica gel composite beads are shown in Fig. 8b, and desorbed epichlorohydrin cross linked chitosan-silica gel composite beads by ethylene diamine tetra acetic acid are shown in Fig. 8b. The change in the scanning electron microscope micrographs of the composite before, and after copper treatment, and desorbed by ethylene diamine tetra acetic acid indicates the structural changes in the composite beads.

Scanning electron microscope pictures of epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads are shown in Fig. 9a , the copper-sorbed epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads are shown in Fig. 9b, and desorbed epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads by ethylene diamine tetra acetic acid are shown in Fig. 9c. The change in the scanning electron microscope micrographs of the composite before and after of copper treatment, and desorbed by ethylene diamine tetra acetic acid indicates the structural changes in the composite beads.

The thermo gravimetric analysis for the epichlorohydrin cross linked chitosan-silica gel composite beads is shown in Fig. 10. The thermogravimetric profiles in Fig. 10 reveal four stages of mass loss. The first and second degradation stage at around 100-228 °C, with a mass loss of 4-5%, is mainly related to the loss of water physically adsorbed on the surface of the materials. In the third degradation stage, mass loss of 10% at 300 °C is seen in Fig. 10. The epichlorohydrin cross linked chitosan-silica gel composite beads undergo a mass loss of 26.6% at 1000 °C.

The thermo gravimetric analysis for the epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads is shown in Fig. 11. The thermo gravimetric profiles in Fig. 11 reveal four stages of mass loss. The first and second degradation stages at around 219.60 °C, with a mass loss of 12.1%, are mainly related to the loss of water physically adsorbed on the surface of the materials. In the second degradation stage, mass loss of 45.0% at 472.07 °C, and the third degradation stage, mass loss of 62.59% at 480.1 °C are seen in Fig. 11. The epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads undergo a mass loss of 100% at
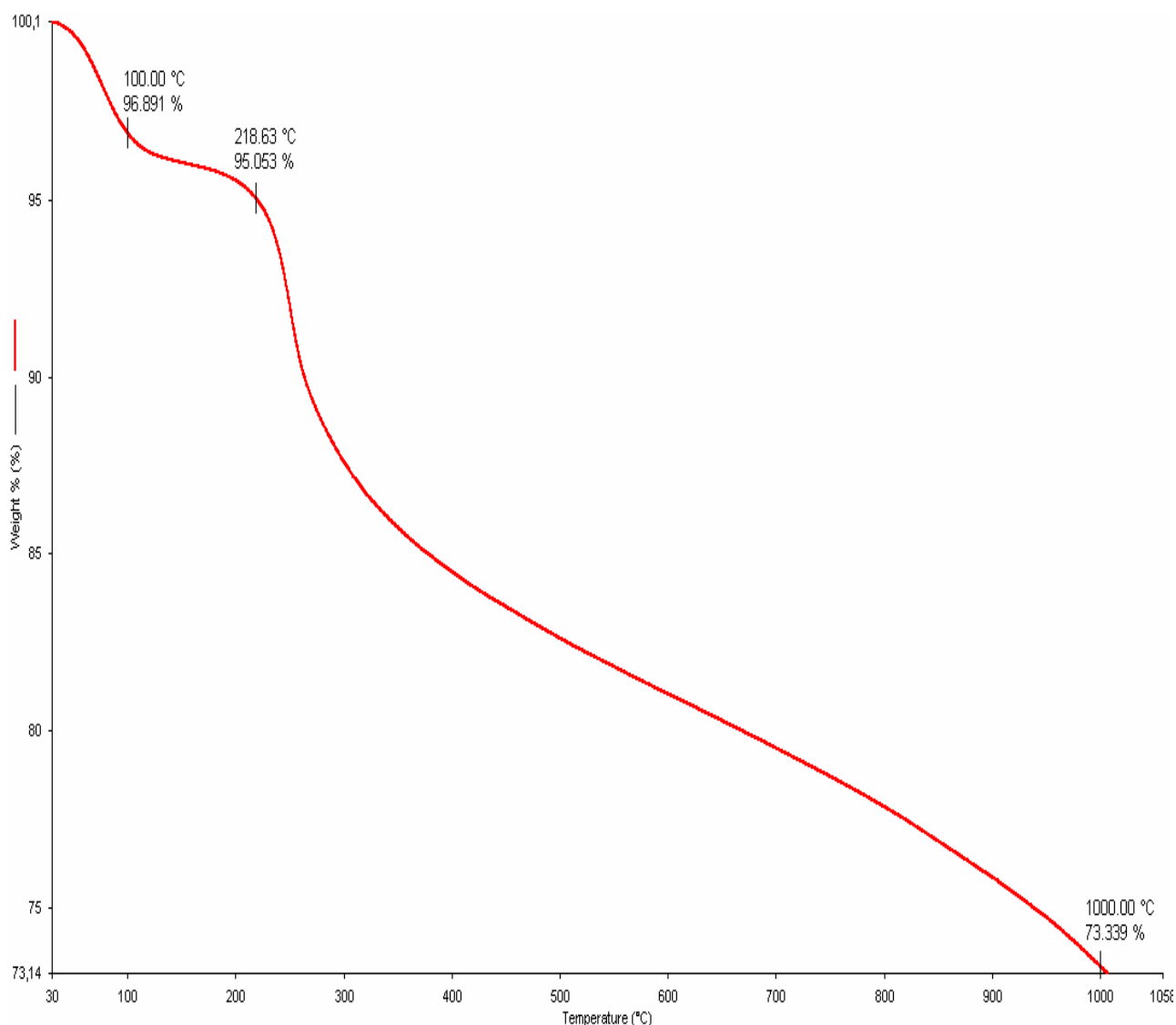
Fig. 10. TGA Analyses of ECH crosslinked chitosan-silicagel composite beads.

1000 °C.

The FTIR spectrums of chitosan, MWCNTs and chitosan MWCNTs composite beads are illustrated in Fig. 12a, adapted from [27]. A band at 3415 cm^{-1} is attributed to -NH$_2$ and -OH group stretching vibration in chitosan. The other peaks of chitosan are C-H stretch peak at 2880 cm^{-1}. The absorption peak at 1422 cm^{-1} is caused by the coupling of C-N stretching vibration. The chitosan and chitosan-composite beads are formed by crosslinking with tripolyphospate, and the peak at 1255 cm^{-1} is attributed to the tripolyphospate ions. The peak at 1077 cm^{-1} corresponds to C-O stretch. The peak at 3648 cm^{-1} in the spectra of MWCNTs is typical to OH groups present on adsorbed water. The absorption peak at 2835 cm^{-1} is attributed to C-H (sp^3). The absorption band at wavenumber 1742 cm^{-1} corresponds to the C-O stretching of carboxylic group on MWCNTs. The peak at 1646 cm^{-1} is attributed to C-H stretching. The peak around 1522 cm^{-1} is assigned to the C-C stretching. The peak at 1063 cm^{-1} is attributed to C-O stretching. The characteristic peaks of the chitosan-MWCNTs composite bead is almost the same as those of the chitosan-MWCNTs. This may be due to the amount of
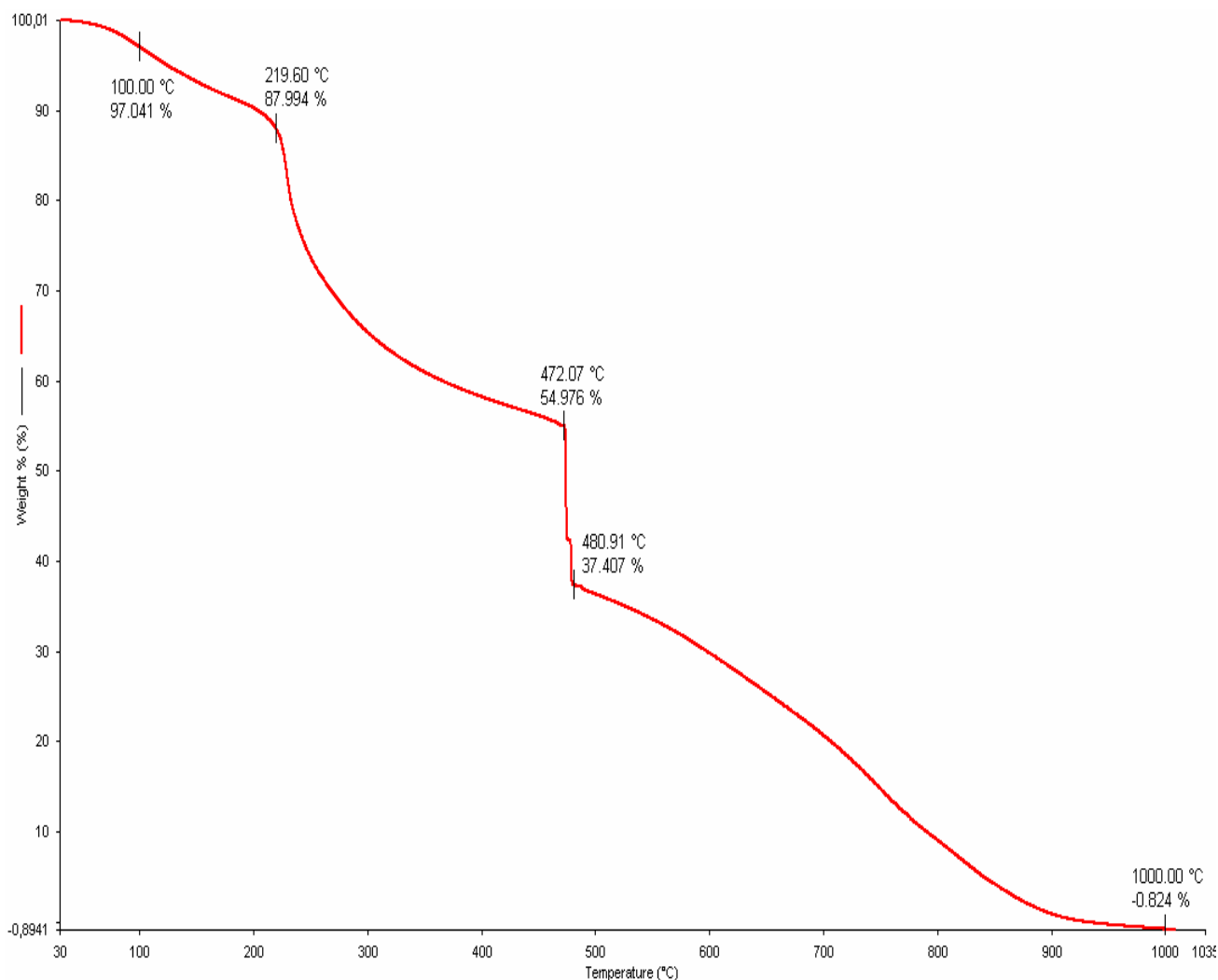
Fig. 11. TGA Analyses of ECH cross-linked carbon nanotube-chitosan composite beads.

MWCNT used and the main chain of chitosan bead is not changed [27].

Figure 13 represents the FTIR spectra of chitosan (a), silicagel (b) and chitosan-silicagel (c) composite adapted from [28]. The bands at 3436, 1110 and 477 cm^{-1} are assigned to the stretching vibration of the surface hydroxyl, the stretching vibration of Si-O and twisting vibration of Si-O-Si, respectively. The major bands for the chitosan can be assigned as follows: 3440 cm^{-1} (OH and NH$_2$ stretching vibrations), 2921 cm^{-1} (CH stretching vibration in CH and CH$_2$), 1652 cm^{-1} (NH$_2$ bending vibration), 1379 cm^{-1} (CH symmetric bending vibrations in CHOH), 1067 and 1028 cm^{-1} (CO stretching vibration in CONH).

CONCLUSIONS

In this comparative study, epichlorohydrin cross-linked silica gel-chitosan composite and epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads were prepared as adsorbent for removing copper(II) ions from aqueous solution. The use of epichlorohydrin cross-linked silica-gel chitosan composite beads and epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads as a preconcentration adsorbent have several advantages; *e.g.,* each adsorbent can be easily prepared, and used at least for three months without changing adsorption properties. Each method described was
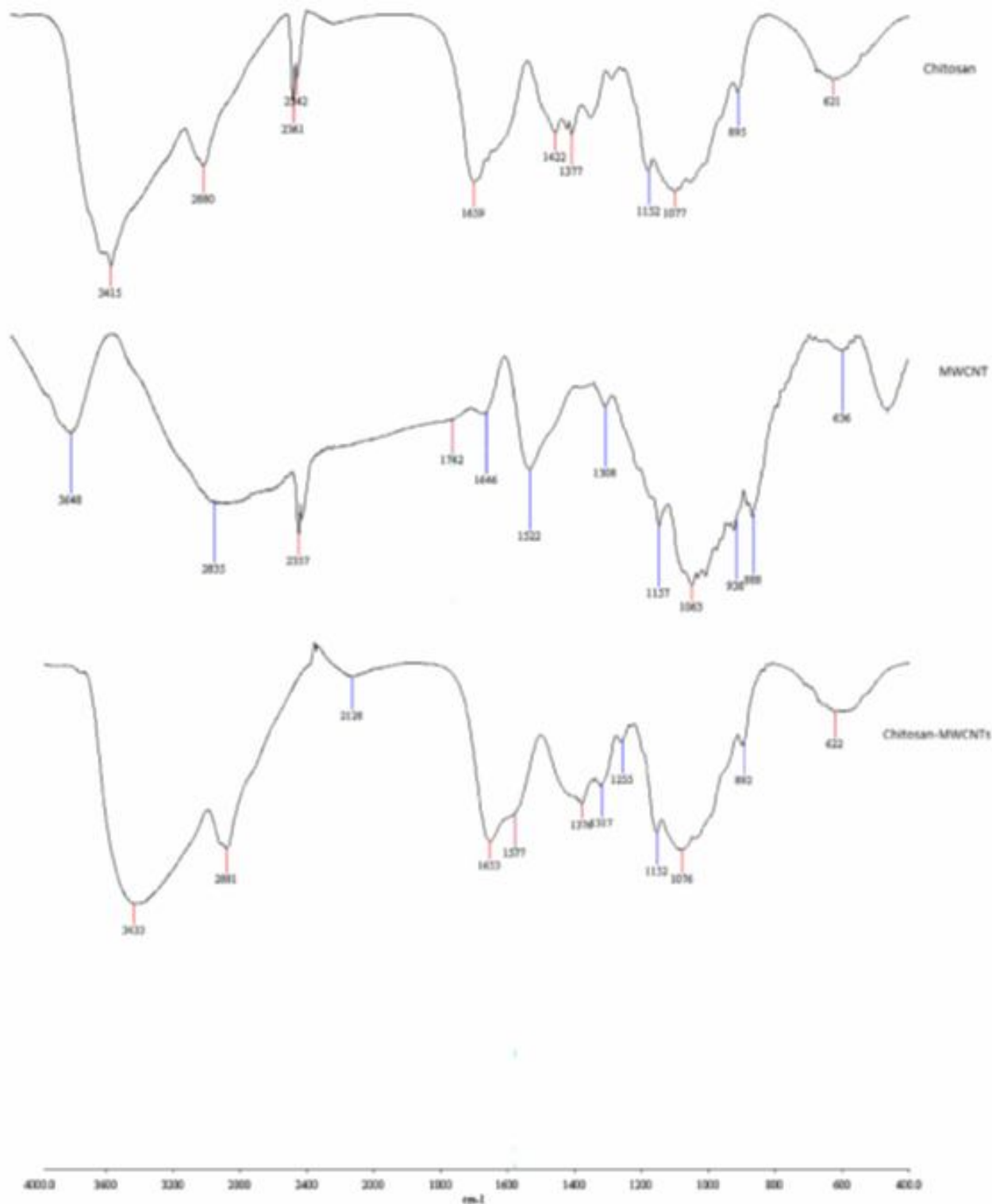
Fig. 12. Infrared spectra of chitosan (a), multiwalled carbonnanotube (b), chitosan-multiwalled carbonnanotube (c) adapted from Ref. [27].
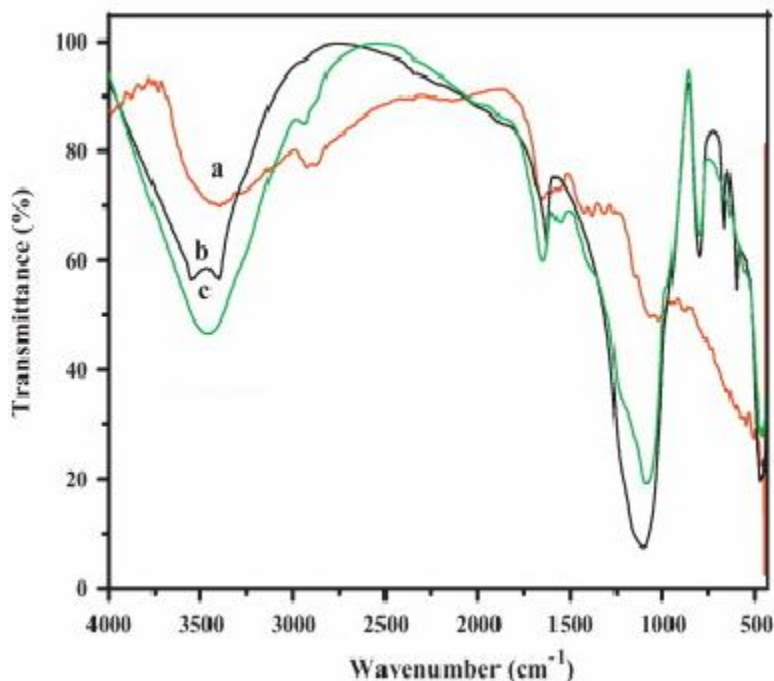
Fig. 13. Infrared spectra of chitosan Chitosan (a) Silicagel (b) Chitosan-Silica-gel (c) adapted from Ref. [28].

successfully used for the preconcentration and determination of copper(II). The elution rate of copper is fast when EDTA epichlorohydrin cross-linked multiwalled carbon nanotube-chitosan composite beads column is used. In order to check the method accuracy, standard reference material was tested for copper in both methods. The results were in good agreement with certified values. In this study, two new adsorbents were developed for online preconcentration of copper, prior to the flame atomic absorption spectrometry. The recovery values obtained for multiwalled carbon nanotube-chitosan composite beads column are higher than those obtained for epichlorohydrin cross linked chitosan-silica gel composite beads column. The system is also very simple, rapid, easy to use, and has a good sensitivity for the determination of Cu(II). In addition, analytical characteristics, such as high enrichment factor, sensitivity and accuracy proved to be promising for the application of the method for trace Cu(II) analysis.

ACKNOWLEDGMENTS

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