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Pre-concentration technique of zinc and copper in water employing dithizone functionalized strong anionic exchange SPE cartridges

David Adeyemi, Solomon Ojigbo, Oluwatosin Johnson and Gloria Ayoola
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy.
University of Lagos, Nigeria.
Idi-Araba Campus, P.M.B. 12003, Lagos, Nigeria.

Corresponding author: David Adeyemi
Email: dkadeyemi@yahoo.com Phone: +2348033871465

ABSTRACT

Background: A simple, selective and rapid method for determining trace amounts of Cu and Zn in water samples using flame atomic absorption spectrophotometry (FAAS) was investigated.

Objective: The objective of this study was to evaluate the efficiency of dithizone functionalized strong anionic exchange (SAX) pre-packed SPE cartridges for pre-concentration of Zn and Cu in water samples, prior to analysis by Flame Atomic Absorption Spectrophotometry (FAAS). This is by focusing on the optimization of recoveries of Zn and Cuin water at varied conditions of pH, volume of sample and eluting solvent.

Materials and Methods: Pre-concentration of metals was by Solid Phase Extraction (SPE) employing dithizone functionalized strong anionic exchange pre-packed cartridges (SAX SPE). Recoveries on SPE were optimized by varying several parameters including pH, volume of sample, volumes of HCl used as eluting solvent and effect of co-retained metal ions.

Results: Recoveries for the unmodified SAX SPE cartridge were 11.41% and 12.90% for Cu and Zn at pH 7.4, when the sample and eluting volumes were 100 mL and 15 mL respectively. The dithizone functionalized SPE when compared to unmodified SPE had improved recoveries for Cu (31.60%) and Zn (29.00%) at optimized conditions of pH 7.4, sample volume, 100 ml, while the eluent volume (HCl) was 20 and 25 mL respectively. The technique of functionalizing SAX pre-parked SPE with dithizone resulted in improved recoveries.

Conclusion: This method could be of great potential for determination of Cu and Zn in water samples, hence warrants further investigation.

Keywords: Copper, Zinc, Dithizone, Solid phase extraction, Flame atomic absorption spectroscopy

Technique de pré-concentration du zinc et du cuivre dans l'eau utilisant des cartouches SPE à échange anionique forte fonctionnalisée avec dithizone

David K. Adeyemi, Solomon O. Ojigbo, Oluwatosin O. Johnson and Gloria A. Ayoola
Département de chimie pharmaceutique, Faculté de pharmacie, Université de Lagos, Nigeria.

Correspondance: David Adeyemi
E-mail: dkadeyemi@yahoo.com Téléphone: +2348033871465

RESUME

Contexte: Une méthode simple, sélective et rapide pour déterminer les traces de Cu et de Zn dans les échantillons d'eau à l'aide d'une spectrophotométrie d'absorption atomique de flamme (SAA-Flamme) a fait l'objet d'une étude.

Objectif: L'objectif de cette étude était d'évaluer l'efficacité des cartouches EPS préemballées à forte concentration anionique (SAX) de dithizone pour la pré-concentration de Zn et Cu dans des échantillons d'eau, avant analyse par Spectrophotométrie d'Absorption Atomique de Flamme (SAA-flamme). Cela, en mettant l'accent sur l'optimisation des récupérations de Zn et de Cu dans l'eau à des conditions variées de pH, volume d'échantillon et solvant éluant.

Méthodes: La pré-concentration des métaux a été effectuée par extraction en phase solide (EPS) à l'aide de cartouches pré-emballées à forte concentration d'anioniques fonctionnalisées par dithizone (SAX SPE). Les récupérations sur EPS ont été optimisées en variant plusieurs paramètres, y compris le pH, le volume d'échantillon, les volumes de HCl utilisés comme solvant éluant et l'effet des ions métalliques conservés.

Résultats: Les récupérations pour la cartouche SAX SPE non-modifiée étaient de 11,41% et de 12,90% pour Cu et Zn à pH 7,4, lorsque l'échantillon et les volumes d'élution étaient respectivement de 100 mL et 15 mL. La dithizone fonctionnelle de l'EPS par rapport à l'EPS non-modifiée a amélioré les récupérations de Cu (31,60%) et de Zn (29,00%) dans des conditions optimisées de pH 7,4, volume d'échantillon de 100 ml, tandis que le volume d'éluant (HCl) était de 20 et 25 mL respectivement. La technique de fonctionnalisation d'EPS pré-emballée SAX avec dithizone a entraîné des récupérations améliorées.

Conclusion: Cette méthode pourrait être d'un grand potentiel pour la détermination du Cu et du Zn dans les échantillons d'eau, justifiant ainsi une étude approfondie.

Mots-clés: Cuivre, Zinc, Dithizone, Extraction en phase solide, Spectroscopie d'absorption atomique de la flamme

INTRODUCTION

Solid phase extraction (SPE) or liquid-solid extraction is a popular and growing technique that is used for sample preparation prior to analysis. It is an attractive alternative for classical liquid-liquid extraction methods that reduce solvent usage, exposure, disposal costs and extraction time for sample separation and concentration purposes. [1, 2] Anion-exchange sorbents for SPE contain weakly basic functional groups such as primary or secondary amines which are charged under low-pH conditions or strongly basic quaternary ammonium groups which are charged at all pHs. [3, 4] Moreover, the SPE disks modified by suitable ligands are successfully used for selective extraction and concentration of metal ions. [5] SPE can easily be used in tandem with Flame Atomic Absorption Spectroscopy (FAAS) without much trouble and is generally considered to be a simple method.[6,7] Dithizone (Diphenylthiocarbazone) is used as a chelating agent for zinc and copper [8] and is widely used for SPE of various metals, since it forms stable complexes with the metals.[9] Some of the disadvantages with SPE cartridges and tubes include columns with a narrow internal diameter limit, the flow rates to a range of 1-10 mL/min that necessitates long trace-enrichment time for large sample volumes. Samples with particulate matter can also plug the cartridge and channelling may occur .[10]

Copper at nearly 40 ng/mL is required for normal metabolism of many living organisms.[11] Copper at trace concentrations acts as a micronutrient in humans and a toxicant in marine and fresh water systems. [12] Copper is also required by plants at very minute levels; beyond this level, it readily binds to cell membrane and hinders the transportation process across the cell wall.

Zinc on the other hand has been known to be an essential trace element for humans, plants and animals. It is a cofactor for more than 300 enzymes, particularly for RNA and DNA polymerases, and for enzymes involved in protein synthesis and cell growth.[13] Zinc deficiency resulting from poor diet, alcoholism and mal-absorption, causes dwarfism, hypogonadism and dermatitis. Metallic zinc has many commercial uses; it has been used as coating to prevent rust, in dry cell batteries and also it has been mixed with other metals to make alloys like brass and bronze. Zinc compounds are widely used in industry to make white paints, ceramics, rubber, wood preservatives, dyes and fertilizers. However, the toxicity of zinc, due to excessive intake, may lead to electrolyte imbalance, nausea and lethargy [14] hence, the widespread use has led to an increase in the levels. As a result, there is need to monitor the levels of Zn and Cu in the environment to safeguard human life.[15] However, the development of a very sensitive pre-concentration technique for determination in sub-micron levels in industrial, medicinal and environmental samples and also for improving the detection limits. Several equipment including electrothermal atomic absorption spectrometry (ETAAS),[16] inductively coupled plasma-mass spectrometry (ICP-MS),[17] and inductively coupled plasma-atomic emission spectrometry (ICP-AES) [18] and flame atomic absorption spectrometry (FAAS) [19] have been employed in analysis of trace metals in samples. **H**istorically, sample pre-concentration was not considered an important factor in sample preparation as emphasis has always been placed on aforementioned instrumentation; there is current need to focus on efficient pre-concentration and clean-up which brings analyte to a suitable concentration level for accurate instrumental analysis. The objective of this study was to evaluate the efficiency of dithizone functionalized strong anionic exchange (SAX) pre-packed SPE cartridges for pre-concentration of Zn and Cu in water samples, prior to analysis by Flame Atomic Absorption Spectrophotometry (FAAS). This is by focusing on the optimization of recoveries of Zn and Cu in water at varied conditions of pH, volume of sample and eluting solvent.

MATERIALS AND METHODS

Apparatus and Reagents

Apparatus include PG990 Flame Atomic Absorption Spectrophotometer (FAAS) equipped with a high intensity hollow cathode lamp and air-acetylene flame (PG instrument, UK); Prep Sep P458 strong anionic exchange (SAX) pre-packed cartridges (Fisher Scientific, USA) coupled to SPE vacuum manifold (Sigma-Aldrich, USA); and the pH meter (Mettler Toledo GMBH, Switzerland). All the reagents used were of HPLC grades, while the solvents were of analytical grade standards (BDH Limited UK) including $ZnSO_4$, $CuSO_4$, deionized water, hydrochloric acid, chloroform and Dithizone.

Preparation of Stock solutions

Standard stock solutions of Zn^{2+} and Cu^{2+} (50mg/L) were each prepared by dissolving 50mg equivalent of the metal ion from $ZnSO_4$ and $CuSO_4$ respectively in deionized water to one Liter. A 50 µg/L solutions of Zn^{2+} and Cu^{2+} were prepared by diluting a proper amount of the stock with deionized water. Dithizone (1.0 g/L) was prepared by dissolving in chloroform, while hydrochloric acid (1.0 mol/L) was prepared by diluting concentrated HCl in deionized water.

Calibration curves

Absorbances of the stock solutions and samples were determined using FAAS. Calibration curve of Absorbance versus concentration for each metal were obtained using Microsoft excel software. The metal ion concentrations in samples were then determined by substitution of absorbance in the regression equation obtained.

Functionalization of SPE sorbent with Dithizone

Each SAX SPE pre-packed cartridge was pre-treated by percolating through with 5 mL of ethanol, 10 mL of water and 5 mL of 1.0 mol/L HCl in succession. Cartridge was then washed with another 10 mL of deionized water in order to remove all the contaminants and then dried by passing air through it for a few minutes. A 3.0 mL of dithizone (1000 mg/L) solution in chloroform was transferred into the cartridge and allowed to penetrate inside the pores of the SPE sorbent. The solvent was allowed to evaporate at 60 ^{o}C for 30 minutes and sorbents dried.[20]

Determination of effect of sample volume on metal recovery

The sample volumes were varied to determine the effect on metal recoveries. Each 100, 150, 200 and 250 mL aliquots of working solutions of Zn^{2+} and Cu^{2+} (50µg/L) were percolated through the dithizone functionalized SAX cartridge at pH 7.4. The metal ions adsorbed on the cartridges were eluted with 15 mL of 1.0 mol/L HCl and the metal ion concentrations were quantified with FAAS. The Instrument settings include: wavelength: 324.8 nm (Cu), 213.9 nm (Zn), Band pass: 0.5nm (Cu), 0.2 nm (Zn), flow rate 1.1 L/min (Cu), 1.2 L/min (Zn), and the signal was continuous at measurement time of 6s. The recoveries were calculated as experimental concentration obtained divided by initial concentration (50 µg/L) expressed as a percentage.

Determination of effect of eluent volume on metal recovery

The eluent volumes were varied to determine the effect on metal recoveries. A 100 mL of each metal ion standards (Zn^{2+} and Cu^{2+}) were percolated through the dithizone functionalized SAX pre-packed cartridge and finally eluted with 1.0 mol/L HCl at different volume of 10 ml, 15 mL, 20 mL and 25 mL. Each metal ion concentration was then determined by FAAS and recoveries calculated.

Determination of effect of pH on metal recovery

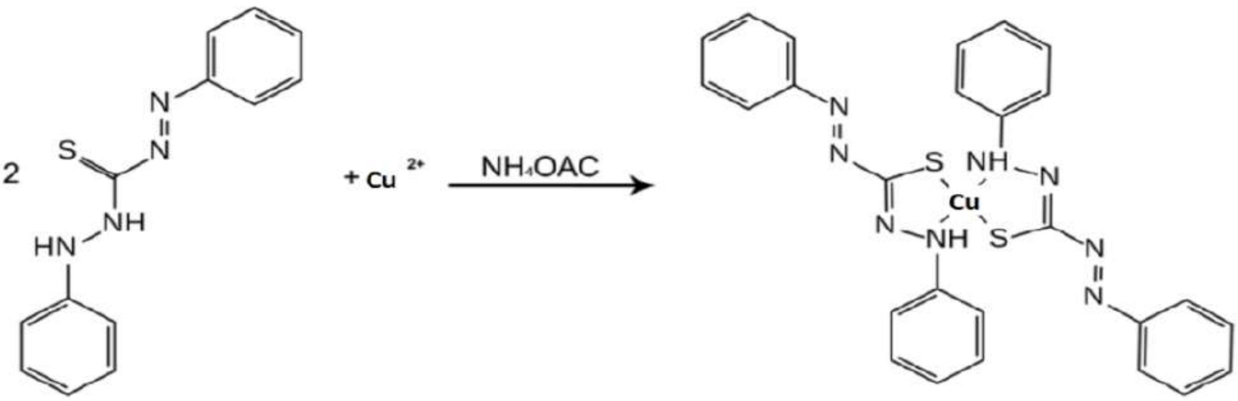
In order to determine the optimized pH, a 100 mL of each working solutions of Zn^{2+} and Cu^{2+} were prepared in quadruplicates and pH of each samples were adjusted to pH 4, 5, 6 and 7 by addition of ammonia and HCl and stabilized with 1.0 M ammonium acetate buffer. The samples were percolated through the dithizone functionalized SAX pre-packed cartridges at a constant flow rate of 5 mL/min. The metal ions adsorbed on the modified cartridge were eluted with 15 mL of 1.0 mol/L HCl and each metal ion concentrations determined with FAAS and the recoveries calculated.

Determination of effect of co-retained compounds on metal recovery

A working solution containing standards mixtures of 50 µg/L of zinc, copper was prepared. A 100 mL of each working solutions was passed through the dithizone functionalized SAX cartridge and eluted with 15mL of 1.0 mol/L HCl and the concentrations of Zn^{2+} and Cu^{2+} were measured by FAAS and the recoveries calculated.

RESULTS

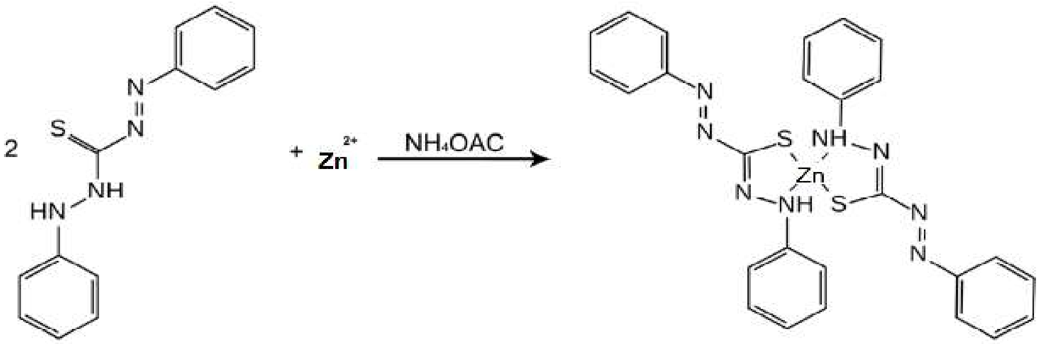
The calibration curve of absorbance versus concentrations for Cu^{2+} and Zn^{2+} standards were linear within the range 0.1 to 1.2 mg/L with the regression equations as $Y=0.139X+0.002$ ($R^2=0.999$) and $Y=0.5959X+0.0057$ ($R^2=0.9993$) respectively. The dithizone functionalized SPE pre-packed cartridges gave better recoveries (Table 1) for Cu (31.6%) and Zn (31.6 and 29.0%) when compared to pre-concentration with unfunctionalized sorbent (11.4 and 12.9%). This result can be attributed to the ability of the dithizone to chelate with Cu^{2+} and Zn^{2+} ions Figure 1and Figure 2 as shown in Figure 1 and 2 below.



Dithizone

Dithizone-Cu coordination complex

Figure 1: Complexation reaction of dithizone with Cu^{2+}



Dithizone

Dithizone–Zn coordination complex

Figure 2: Complexation reaction of dithizone with Zn^{2+}

The critical volume of Zn^{2+} and Cu^{2+} solutions was 100.0 mL, above this value, there was decline in recovery (Figure 3) and this corroborates the results of previous studies where 100 mL was the volume of sample loaded on the adsorption sorbent for optimum recoveries.[20]

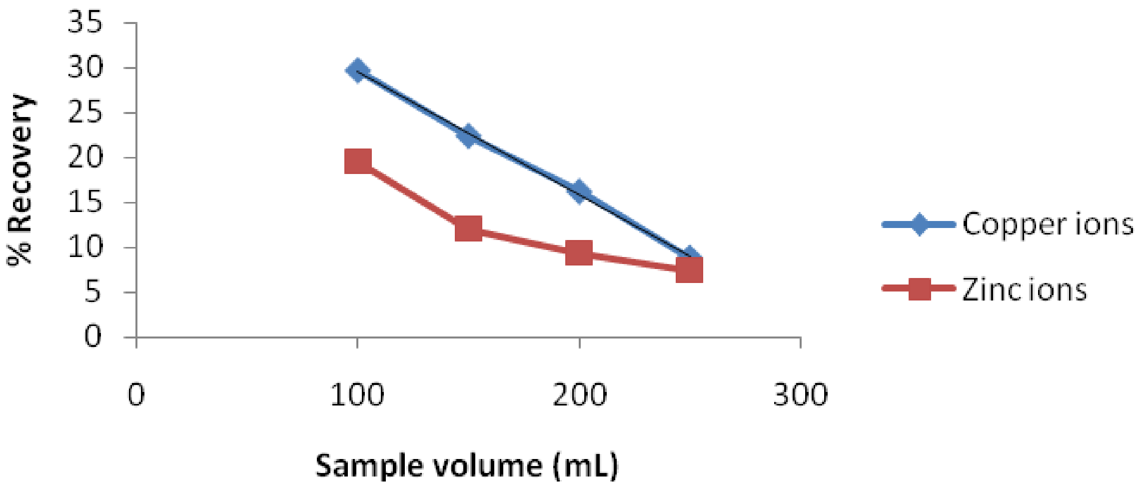


Figure 3: Variation of recoveries of Cu^{2+} and Zn^{2+} from dithizone functionalized C-18 SAX cartridge with sample volume (conditions: 3µg, sample flow rate: 5 mL/min, pH 7.4 and eluent volume: 15 mL)

DISCUSSION

Effect of sample volume, eluent volume and pH on Recovery studies

Dithizone is a bidentate chelating agent and possesses both sulfur and nitrogen coordination site which could donate an electron pair to metal ions, hence, forms stable chelating complex with metal ions, thus acting as a Lewis base. When the volume of acid or concentration of hydronium ion was increased, the nitrogen site which is more susceptible to protonation is unable to bond to metal ions and hence retention is reduced.

Due to the intrinsic properties of the coordination complex formed between the dithizone and the divalent metal ions, an acidic solution was employed for desorption of the ions from the metal chelates. Hydrochloric acid is an effective eluting solvent because it has the ability to protonate the chelating sites and elute cartridge of Zn^{2+} and Cu^{2+} ions. The volume of eluent (HCl) was optimized in this study and recovery was optimal when the eluent volumes were in the range 10 mL and 25 mL. There were no considerable increase in recovery for the eluent volume greater than 25 and 20 mL for Zn^{2+} and Cu^{2+} respectively (Figure 4).

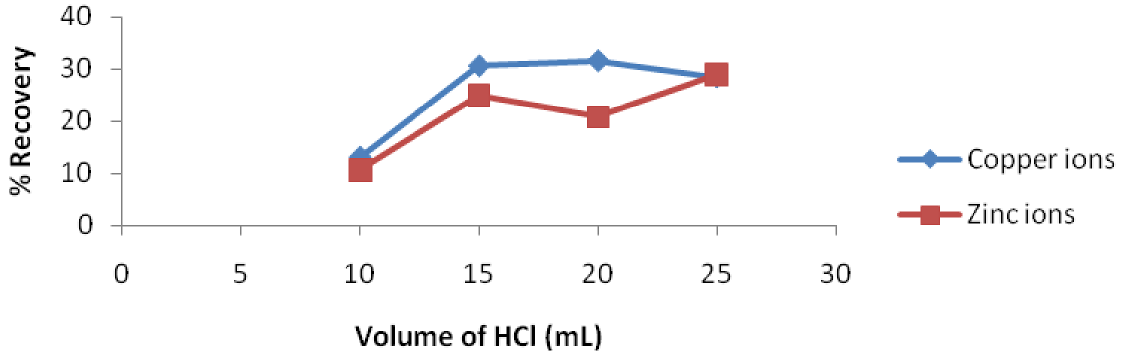


Figure 4: Variation of recoveries of Cu^{2+} and Zn^{2+} from dithizone functionalized C-18 SAX cartridge with eluent volume (conditions: 3µg, flow rate: 5 mL/min, pH 7.4 and sample volume: 100 mL)

The adsorption of Cu^{2+} and Zn^{2+} occurs as a result of formation of a coordination compound. Since pH has a profound effect on the retention of the ions by the ligand, it is another important parameter influencing SPE process. At low pH, the metal ions are present as the hydrated form, while the metal hydroxo species becomes more predominant at higher PH.[21] Increasing the pH of the sample would promote the Lewis character of dithizone ligand, and at low pH the extra acid may hydrolysed and destroy the solid support which is probably why the optimum recovery of Cu^{2+} and Zn^{2+} (Figure 5) from water samples was obtained at pH 8.

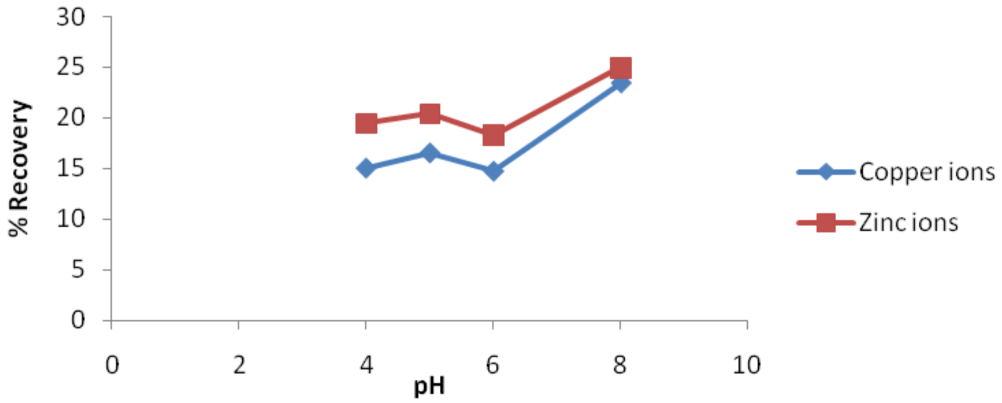


Figure 5: Variation of recoveries of Cu^{2+} and Zn^{2+} from dithizone functionalized C-18 SAX cartridge with pH (conditions: 3µg, sample flow rate: 5 mL/min, eluent volume: 15 mL; sample volume: 100 mL)

Effect of co-extraction of metal ions on analyte recovery

The relative affinity of metal ions for dithizone ligand is an important factor affecting selective recovery of a particular metal ion in the presence of other ions. To investigate this effect, a 100 mL aliquot of working solution containing 50µg/L of zinc and copper was passed through the dithizone functionalized SAX cartridge. The recoveries of Cu (10.51%) and zinc (18.02%) in the presence of co-retained metal ions were relatively lower at optimized conditions (Table 1) when compared to when there is no interference of co-extraction

Table 1: Absorbance, concentration and % recoveries of Cu^{2+} and Zn^{2+} on unfunctionalized SPE pre-packed cartridges at stated conditions of pH, eluent and sample volume

Parameters	Copper	Zinc
pH	7.4	7.4
Eluent volume (m L)	15	15
Sample volume (m L)	100	100
Absorbance	0.003	0.018
Concentration (µg/L)	0.038	0.043
% Recovery	11.4	12.9

The relatively low recoveries may be as a result of the competition of the various metal ions in the sample matrix for the dithizone ligand binding site.[22] The results also indicate that the binding or stability constant for the formation of Zinc- dithizone complex at the investigated conditions may be higher than that of copper.

It is recommended that future studies be focused at optimizing the metal recoveries employing dithizone functionalized strong cationic exchange SPE sorbent for comparative studies. It should also aim at application of developed technique in determining the mean concentrations of metal ions in real water samples.

CONCLUSION

The study effectively compared efficiencies of dithizone as a chelating ligand in solid phase extraction of metals. Functionalization of the strong anionic exchange pre-packed SPE with the dithizone improved recoveries for zinc and copper ions.

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REFERENCES

1 Bulut Y, Tez Z. (2007). Removal of heavy metals from aqueous solution by sawdust adsorption. *Journal of Environmental Science* 19(2): 160–166.

2 Mohammadi SZ, Afzali D, Pourtalebi D. (2011). Flame atomic absorption spectrometric determination of trace amounts of palladium, gold and nickel after cloud point extraction. *Journal of Analytical Chemistry* 66(7): 620–625.

3 Silva EL, Ganzarolli EM, Carask E. (2004). Use of Nb_2O_5-SiO_2 in an automated on-line preconcentration system for determination of copper and cadmium by FAAS. *Talanta* 62, 727-733.

4 Tzvetkova P, Nickolov R (2012). Modified and unmodified silica gel used for heavy metal ions removal from aqueous solutions. Journal of the University of Chemical Technology and Metallurgy 2012; 47(5), 498-504.

5 Shamsipur M, Ghiasvand AR, Sharghi H. (2002). Preconcentration of Ultra Trace Hg(II) in Aqueous Samples on Octadecyl Silica Membrane Disks Modified by Dibenzodiazathia-18-Crown-6-Dione and Its Determination by Cold Vapor Atomic Absorption Spectrometry. *International Journal of Environmental and Analytical Chemistry* 82: 23-30.

6 Zh L, Changa X, Zoua X, Niea R, Zh H and Lia R. (2009). Chemically-Modified Activated Carbon with Ethyl-enedia-Mine for Selective Solid-Phase Extraction and Preconcentration of Metal Ions. *Analytica Chimica Acta* 632 (2): 272-277.

7 Burham N, Abdel-Azeem SM, El-Shahat F. (2008). Determination of Lead and Cadmium in Tap Water and Ap-ple Leaves after Preconcentration on a New Acetylace-tone Bonded Polyurethane Foam Sorbent. *International Journal of Environmental Analytical Chemistry* 88 (11): 775-789.

8 Akira S, Masahide Y, Hiroshi Y, Hiroshi F, Shigeaki I, Kouko T, Yoshiko T. (2002). Identification of insulin-producing cells derived from embryonic stem cells by zinc-chelating dithizone. *Stem Cells* 20(4): 284 292.

9 Manzoori JL, Karim-Nezhad G. (2003). Selective cloud point extraction and preconcentration of trace amounts of silver as a dithizone complex prior to flame atomic absorption spectrometric determination. *Analityca Chimica Acta* 484(2): 155-161.

10 Ahalya N, Ramachandra TV, Kanamadi RD. (2003). Biosorption of heavy metals. *Research Journal of Chemistry and Environment* 7:71-78.

11 Govindaraju M, Shekar HS. (2011).The role copper in clinics of neurological disorder. *International Journal of Research in Pharmacy and Chemistry* 1: 320–328

12 Croot PL, Moffett JW, Brand LE. (2000). Production of extracellular Cu complexing ligands by eucaryotic phytoplankton in response to Cu stress. *Limnology and Oceanography* 45:619- 627.

13 Kazi T, Wadhwa SK, Afridi HI, Kazi N, Kandhro GA, Baig J.A, Shah AQ, Kolach NF. (2010). Interaction of cadmium and zinc in biological samples of smokers and chewing tobacco female mouth cancer patients. *Journal Hazardous Materials* 176: 985-991.

14 Storelli MM, Barone G, Storelli A, Marcotrigiano GO. (2006). Trace metals in tissues of Mugilids (Mugil auratus, Mugil capito and Mugil labrosus) from the Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology* 77: 43-50.

15 Jimoh T, Egila JN, Dauda BEN, Iyaka YA. (2011). Preconcentration and removal of heavy metal ions from aqueous solution using modified charcoal. *Journal of Environmental Chemistry and Ecotoxicology* 3(9): 236-241.

16 Acar O. (2004). Determination of lead, copper, manganese and zinc in botanic and other biological samples by electrothermal atomic absorption spectrometry using scandium- containing chemical modified. *Analytical Chimica Acta* 526: 103 -109.

17 Nomizu T, Hayashi H, Hoshino N, Tanaka T, Kawaguchi H, Kitagawa K, Kaneco S. (2002). Determination of Zinc in Individual Airborne Particles by Inductively Coupled Plasma Mass Spectrometry with Digital Signal Processing. *Journal of Analytical Atomic Spectrometry* 17: 592–595.

18 Sreenivasa Rao K, Balaji T, Prasada Rao T, Babu Y, Naidu GRK. (2002). Determination of iron, cobalt,

nickel, manganese, zinc, copper, cadmium and lead in human hair by inductively coupled plasma-atomic emission spectrometry," *Spectrochimica Acta B* 57(8): 1333–1338.

19 Dutra RL, Maltez HF, Carasek E. (2006). Development of an on-line preconcentration system for zinc determination in biological samples. *Talanta* 69(2): 488 -493.

20 Davarani SSH, Sheijooni-Fumani N, Najarian AM, Mohammad-Ali T, Vahidi S. (2011). Preconcentration of Lead in Sugar Samples by Solid Phase Extraction and Its Determination by Flame Atomic Absorption Spectrometry, *American Journal of Analytical Chemistry* 2:626-631.

21 Matoso E, Kubota LT, Cadore S. (2003). Use of silica gel chemically modified with zirconium phosphate for preconcentration and determination of lead and copper by flame atomic absorption spectrometry. *Talanta* 60: 1105-1111.

22 Shao-Song Qian , Hai-Hua Li , Hui Zhu , Zhong-Ming Yang , Zhong-Lu You & Hai-Liang Zhu (2013). Syntheses, Crystal Structures, Thermal Stability and Fluorescence Properties of Zinc (II) Complexes With Tridentate Schiff Bases. *Journal of Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry* 43(4): 412-416