

INVESTIGATION OF DITHIZONE MODIFIED C-18 SPE SORBENTS FOR PRE-CONCENTRATION OF Zn AND Cu IN WATER SAMPLES

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ABSTRACT

A simple and sensitive solid phase extraction of Zn^{2+} and Cu^{2+} in water samples employing Dithizone modified octadecyl (C-18) SPE sorbents was investigated. The effect of Phenanthroline and EDTA chelation of metals on extraction efficiencies of sorbents was compared for unmodified and dithizone modified C-18 sorbents. Each C-18 SPE sorbent was pre-treated, and 100 ml of each sample of Zn^{2+} and Cu^{2+} (100 μ g/L) at different pHs (3.0, 5.0, 7.5 and 10) was percolated through the dithizone modified C-18 SPE sorbent at a steady flow rate of 5ml/min. The procedure of metal chelation was by mixing 100 ml of sample each with 2ml of EDTA and phenanthroline ligands (1000 mg/L) at varied pH and percolating through the C-18 pre-packed cartridge. The adsorbed metal ions were eluted with 5.0 mL of hydrochloric acid (1.0 mol/L) and concentrations were determined by flame atomic absorption spectrometry FAAS. Recoveries from the sorbents were evaluated and expressed in percentage.

Recoveries were generally low (0.0-28.5%) for Cu^{2+} at the investigated pHs. Dithizone modified C-18 SPE gave better recoveries for 66.7 % of Zn samples but lower recoveries for Cu in 41.7 % of samples, when compared to the unmodified sorbent. SPE technique based on EDTA-Zn chelation resulted in optimum recovery (90.0%) for Zn at pH 3, while phenanthroline-Zn chelation produced optimum recovery (31.1%) at pH 10. The dithizone modified C-18 SPE sorbent produced better recoveries for Zn^{2+} from water samples when compared to unmodified one and could be a potential technique of sample preparation for determination of metals in water samples, hence warrants further investigation.

Keywords: Solid Phase Extraction, ligands, modified, sorbent, recoveries

INTRODUCTION

Solid phase extraction (SPE) is an attractive technique that reduces solvent usage and exposure, disposal cost and extraction time for sample preparation (Marahel *et al.*, 2011; Khayatian *et al.*, 2010). Recently a great deal of work has been devoted to SPE as a pre-concentration technique. It offers advantages such as short extraction time, low cost, high enrichment factors, recoveries and low consumption of non environment-

friendly solvents. 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 (Zh *et al.*, 2009; Burham *et al.*, 2008). The most pivotal step in SPE is considered to be choosing the sorbent material as it gives the method its characteristic properties such as selectivity and capacity towards various metal ions. C-18 are still widely used as a sorbent, however, due to its limited ability to absorb

metal ions quantitatively at trace and ultra-trace levels, its surface is treated with chelating agents. Dithizone (Diphenylthiocarbazone) is used as chelating agent for zinc (Akira *et al.*, 2002) and is widely used for the liquid-liquid and Solid phase extraction of various metals (Manzoori and Karim-Nezhad, 2003) and is used for modification of sorbent in this work. 1,10-Phenanthroline, a conventional chelator, was also used to study its efficacy (Mitsuhiro *et al.*, 2014). Ethylenediaminetetraacetic acid is a chelating agent and readily forms complexes with rare earth metal ions and the stability constants of these complexes have been evaluated (Giorgio *et al.*, 2004). 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 (Yamini and Tamaddon, 1999).

Copper at nearly 40 ng/mL is required for normal metabolism of many living organisms (Burtis and Ashwood, 1999). Copper at trace concentrations acts as micronutrient in marine and fresh water systems (Taylor and Anstiss 1999; Croot *et al.*, 2000). It is required by plants at only very minute levels, at higher levels, copper can be bound to the cell membrane and hinders the transport process through 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 (Iwata *et al.*, 1999). 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 useful in industries to make white paints, ceramics, rubber, wood preservatives, dyes and fertilizers. However, the widespread use can lead to the contamination of the environment and excessive exposure to humans can result in electrolyte imbalance, nausea and lethargy (Storelli *et al.*, 2005). Hence, there is need to monitor the levels of Zn and Cu in the environment to safeguard human life (Lau and Cheng, 1998). The development of a very sensitive pre-concentration technique for determination in sub-micron levels in industrial, medicinal and environmental samples and also for lowering the detection limits for accurate instrumental analysis is very important. Several detectors including electrothermal atomic absorption spectrometry (ETAAS) inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) and flame atomic absorption spectrometry (FAAS) have been employed in analysis of trace metals in samples (Acar 2004, Nomizu 2001, Dutra 2006, Montaser and Golightly, 1999). In current study, a simple and sensitive solid phase extraction of Zn^{2+} and Cu^{2+} in water samples employing Dithizone modified octadecyl (C-18) SPE sorbents was investigated. The effect of Phenanthroline and EDTA chelation of Zn^{2+} and Cu^{2+} on extraction efficiencies of sorbents was also compared for unmodified and dithizone modified C-18 sorbents. Each metal ion in water samples was analysed with FAAS and recoveries were determined at different pH.

MATERIALS AND METHODS

Apparatus

Quantification of Zn and Cu contents in water samples were by a PG990 Flame Atomic Absorption Spectrophotometer (FAAS) equipped with a high intensity hollow cathode lamp and an air-acetylene flame according to the recommendations of the manufacturers (PG instrument, UK). Pre-concentration was by using C-18 pre-packed SPE (1141417) sorbent (500 mg) equipped with filtration apparatus and vacuum manifold (Sigma-Aldrich, USA), while the pH was measured with pH meter (Mettler Toledo, Seven Easy pH Switzerland).

Reagents

Distilled deionized water and analytical grade chemicals (ethanol, hydrochloric acid, chloroform, dithizone, EDTA, Phenanthroline, ammonium acetate, acetic acid) were used throughout the experiments. All solvents used were of HPLC grade and were purchased from BDH Limited UK. Standard stock of Zn and Cu (50 mg/L) were each prepared by dissolving 50 mg equivalent of the metal ion from $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ with deionized water respectively, while working solutions of Zn and Cu was prepared by diluting a proper amount of the aforementioned solutions. Dithizone (1.0 g/L) was prepared by dissolving in chloroform, while hydrochloric acid (1.0 mol/L) was prepared by dilution of the stock in deionized water.

Preparation of stock and working solutions

A stock solution of Zn^{2+} (50mg/L) was prepared by dissolving 220.15mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ in deionized water and diluted to one litre, while a stock solution of Cu^{2+} (50mg/L) was prepared by dissolving 125.5 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in deionized water and

diluted to a litre. The working solutions (100µg/L) of Zn^{2+} and Cu^{2+} were each prepared by taking 2.0 ml of the stock in a standard flask and making up to 1.0 Litre mark. The working solutions were prepared in four portions and the pH were adjusted to different values (3, 5, 7.5 and 10) by addition of NH_3 and HCl and stabilized with 1.0 M ammonium acetate buffer.

Analytical Procedures

Each C-18 SPE sorbent was pre-treated according to the method of Saied et al., 2011. This is by percolating through the sorbent with 5.0 mL of ethanol, 10.0 mL of water and 5.0 mL of 1.0 mol/L HCl. Cartridge was then washed with another 10.0 mL of deionized water in order to remove all the possible contaminants during the manufacturing process and then dried by passing air through it for few minutes. In preparing a Dithizone modified C-18 SPE sorbent, a 2.0 mL of Dithizone (1000 mg/L) solution in chloroform was poured in to the cartridge and allowed to penetrate in to the pores of the SPE sorbent (Saied et al., 2011). A 100 ml of each sample of Zn^{2+} and Cu^{2+} (100µg/L) at different pH was percolated through the dithizone modified C-18 SPE sorbent at a steady flow rate of 5ml/min using a suction pump and thereafter eluted with 5.0 mL of 1.0 mol/L hydrochloric acid. The SPE procedure for metal chelation was by mixing 100 ml of each prepared samples with 2ml of EDTA and phenanthroline ligands (1000 mg/L) at different pH (3.0, 5.0, 7.5 and 10) and percolating through the C-18 pre-packed cartridge at a flow rate of 5ml/min. The adsorbed metal ions were eluted with 5.0 mL of 1.0 mol/L hydrochloric acid and each metal was quantified with FAAS.

RESULTS AND DISCUSSION

Recoveries study

The percent recoveries were each determined by evaluating eluted concentration divided by the spiked concentration expressed as a percentage. Recoveries of unchelated Zn^{2+} at varied pH (3, 5, 7.5, and 10) for the unmodified C-18 sorbent were 39, 1.6, 10.1 and 5.8 % respectively (Table 1), this results revealed that the retention capacity of the sorbent was best favoured by the acidic condition of pH 3, while the recoveries of unchelated Cu^{2+} from the unmodified C-18 sorbent were generally low and ranged from 1.0 to 4.4 % (Table 2). The retention of analytes from polar solutions (e.g. water) onto the SPE material has been attributed primarily to non-polar attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the silica surface (Żwir-Ferenc *et al.*, 2006). These attractive forces include Van Der Waals forces, or dispersion forces. The recoveries of the unchelated Zn^{2+} from the dithizone modified C-18 SPE cartridge were 38.6, 5.4, 13.7 and 7.6 % at pH 3, 5, 7.5 and 10 respectively

(Table 1), while the recoveries of unchelated Cu^{2+} from the dithizone modified C-18 SPE cartridge was 9.2, 28.5, 10.0 and 22.9% at pH 3, 5, 7.5 and 10 respectively (Table 2). In this study, recoveries were best favoured at pH 3 and 5 for Zn^{2+} and Cu^{2+} respectively on modification with dithizone. Dithizone has two active hydrogen atoms that can be substituted by a cation; hence, it has a strong affinity for the ions of the *d*-electron transitive elements to form neutral complexes (Chwastowska *et al.*, 2007). After modifying the C-18 cartridge with dithizone, the tendency to retain Zn^{2+} increased considerably at pHs 5, 7.5 and 10 when compared with unmodified one.

The unusual property of deprotonated *form* of Ethylene diamine tetraacetic acid (EDTA) is its ability to chelate metal ions in 1:1 metal-to-EDTA complexes. The EDTA ion being a hexadentate ligand entirely wraps up divalent transitive metal ions such as Zn^{2+} and Cu^{2+} to form a negatively charged complex species with the co-ordination number of six.

Table 1: % Recoveries of Zn^{2+} at varied pH

pH	Volume of sample	Volume of eluent	Unchelated Zn^{2+} solution on unmodified C-18 SPE	Unchelated Zn^{2+} solution on Dithizone modified C-18 SPE	EDTA – Zn^{2+} chelated solution on dithizone modified C-18 SPE	1,10-Phenanthroline - Zn^{2+} solution on dithizone modified C-18 SPE
	(ml)	(ml)	Percentage recoveries (%)			
pH 3	100	5.0	39.00	38.6	90.0	1.5
pH 5	100	5.0	1.60	5.4	0.8	29.0
pH7.5	100	5.0	10.05	13.7	8.3	27.2
pH10	100	5.0	5.80	7.6	24.0	31.1

Table 2: % Recoveries of Cu²⁺ at varied pH

pH	Volume of sample	Volume of eluent	Unchelated Cu ²⁺ solution on unmodified C-18 SPE	Unchelated Cu ²⁺ solution on Dithizone modified C-18 SPE	EDTA–Cu ²⁺ chelated solution on dithizone modified C-18 SPE	1,10-Phenanthroline - Cu ²⁺ chelated solution on dithizone modified C-18 SPE
	(ml)	(ml)	Percentage recoveries (%)			
pH 3	100	5.0	1.0	9.20	0.0	0.0
pH 5	100	5.0	4.4	28.5	0.4	1.2
pH 7.5	100	5.0	1.1	10.0	0.2	1.4
pH 10	100	5.0	1.1	22.9	0.4	0.0

In this study, the addition of 2ml of EDTA solution to the metal ion allowed the formation of metal complex prior to extraction. The results confirmed that the EDTA –metal complex shows the optimum recovery (90.0%) for Zn²⁺ at pH 3 (Table 1). This corroborates the report of Kovacevic *et al.*, 2002 that EDTA has good affinity for divalent metal ions under acidic conditions, but poor affinity for metal ions under neutral and alkaline conditions. It has been reported that EDTA competes with organic ligands for Cu complexation, but its Cu chelate is not easily retained by the C-18 cartridges. This is because Cu does not form a stable complex with EDTA and hence, the chelation in this study appears to reduce the sorption efficiency of Cu by the SPE cartridge (Dalman and Karabocek, 2003). This is probably a possible reason for poor recoveries of Cu (0-0.4%) in this study.

1, 10-phenanthroline is a heterocyclic organic compound used as a ligand in coordination chemistry, and binds to metals ions more tightly to form a positively charged complex (Sílvia *et al.*, 2016). In current study, quantitative retention of sorbent in the presence of 1, 10-phenanthroline produced the optimum

recovery for Zn²⁺ (31.1%) at pH 10 (Table 1), and generally low recoveries for Cu²⁺ ($\leq 1.4\%$) at investigated pH values (Table 2).

The equilibrium process in metal chelating is largely affected by several factors which often strengthen or weaken the chelates, including ionic strength, solvent effects as well as pH. Of these, pH has been the most extensively studied factors (Deacon *et al.*, 1994). In pH range of aqueous solution, most of the well-known chelating agents including EDTA exist as an equilibrium mixture of both protonated and unprotonated forms (Yu *et al.*, 1994). In this study, the maximum recoveries of Zn (90.0 %) and Cu (28.5%) from water samples were obtained at pH 3 and 5 respectively. This confirms that the metal ion has a high affinity for EDTA in acidic medium, and at this condition; metal ions compete effectively with H⁺ ions for the available donor atoms. The form of metal ion in solution changes according to pH level. At low pH values, the metal ions prefer the hydrated form, but the metal hydroxo species becomes more predominant as pH increases. 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 (Shaw *et al.*, 2003).

CONCLUSION

The proposed SPE technique based on dithizone modified octadecyl silica sorbent produced improved recoveries and could be a precise technique for the determination of Zn^{2+} and Cu^{2+} in water samples and hence warrants further investigation.

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