

# Application of Solid-Phase Extraction in Metal Speciation

Isai T. Urasa\*, Stephen F. Macha, and Weam El-Maaty

Department of Chemistry, Hampton University, Hampton, VA 23668

## Abstract

Solid-phase extraction materials that consist of C<sub>18</sub> and amino (LC-NH<sub>2</sub>) functionalities are used to extract metal species in aqueous solutions. The C<sub>18</sub> is employed after the metal species are converted into a neutral complex with dithizone. The complex is then eluted from the C<sub>18</sub> substrate by using an eluent that consists of acetonitrile in nitric acid medium. Although the C<sub>18</sub> material is found to be suitable for the extraction of neutral metal complexes, the LC-NH<sub>2</sub> material is applicable to the direct extraction of free metal species. Moreover, because the NH<sub>2</sub> functionality is amenable to protonation, sample acidity has a significant influence on the nature and effectiveness of the extraction process. In general, solid-phase extraction immobilizes metal species on the solid substrate, thereby maintaining its stability. This is found to be particularly true for the neutral metal complexes extracted on the C<sub>18</sub> material. Dithizone metal complexes are generally quite unstable under normal atmospheric conditions. When the complex is extracted and left on the solid substrate, however, its stability is unchanged. Closely related to species stability is sample matrix, the effects of which are completely eliminated by immobilizing the analyte on the solid substrate. The separation of the analyte from the sample matrix also facilitates its preconcentration. Measurement sensitivity is increased several factors by the extraction of several aliquots of dilute solutions of the analyte.

## Introduction

Solid-phase extraction (SPE) has been used extensively in the last 15 years as an alternative to liquid-liquid extraction in the analysis of environmental samples for organic compounds. This method has several advantages over liquid-liquid extraction. First, it uses fairly small sample amounts. Second, the analyte of interest can be completely isolated from the complex sample matrix in a single operation. Third, sample preconcentration by several orders of magnitude can be achieved. Fourth, by selecting solid-phase devices with specific functional groups, the desired

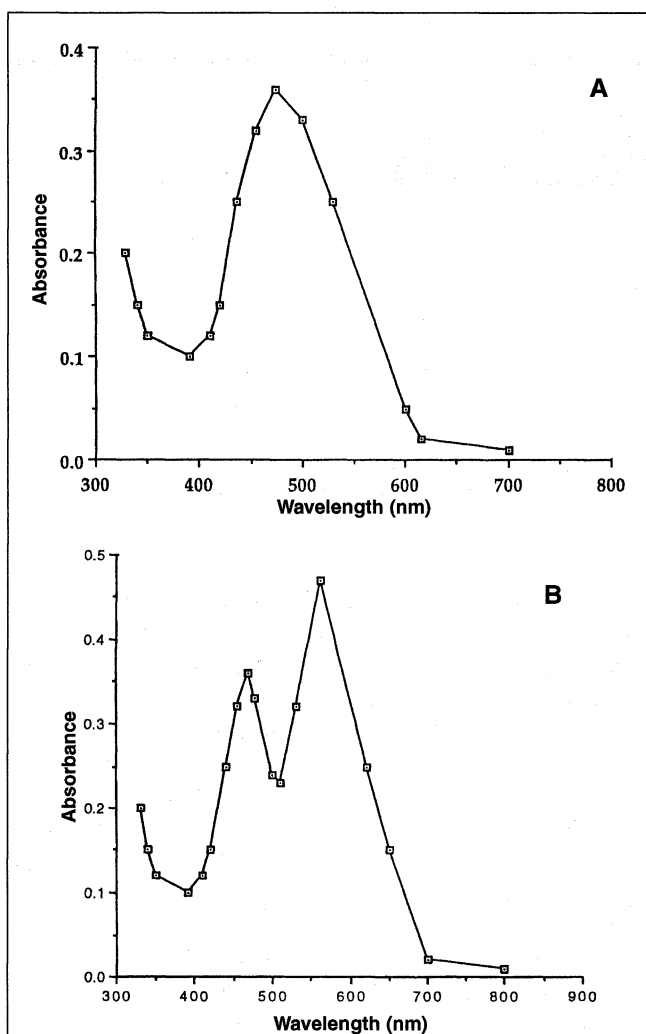
compounds or group of compounds can be preferentially separated from the bulk sample. However, although SPE has been widely applied in environmental analysis of organic compounds, its application in metal speciation has been relatively rare.

Metal species in solution can exist as free hydrated ions, neutral complexes, or charged complex ions. Though the classical liquid-liquid extraction can be used for chemical species that are soluble in organic solvents, it is not as effective for the inorganic metal fractions. The inherent equilibria that come into play during ligand dissociation, formation of complex compounds with the metal ions in solution, and the subsequent migration of the complex into the organic phase make the extraction process cumbersome and inefficient. Furthermore, when ligand properties are considered, it is found that the process can be even more complicated. Ligands such as dithizone interact with metal ions to form neutral complex species. Others, such as 1,10-phenanthroline, form positively charged complex ions, whereas ethylenediaminetetraacetic acid (EDTA) and similar polycarboxylic ligands generally form negatively charged complex species. In any given natural or waste water system, it is expected that a combination of these ligands will be present from natural or man-made sources. Thus, the speciation of metals in samples of this nature can be severely limited if the liquid-liquid extraction approach is employed.

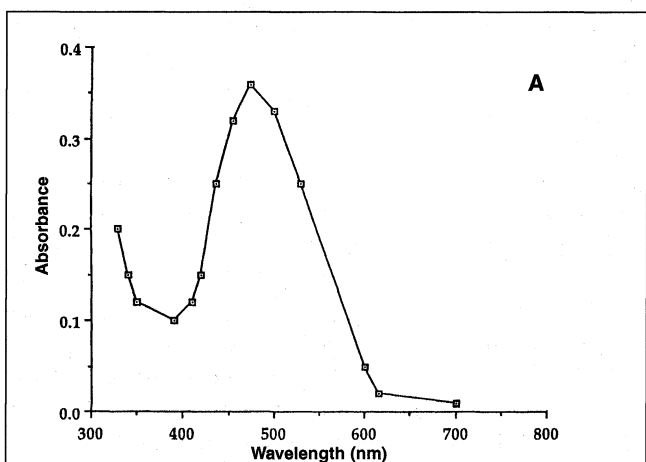
By using SPE, the limitations inherent in the multi-equilibrium liquid-liquid extraction process are circumvented. This is because SPE uses sorbents that consist of specific functional groups immobilized on solid support. The extraction process is governed solely by the interaction of the analyte and the functional group on the SPE substrate. Moreover, the sorbent can be fitted with a specific functional group for removing specifically desired chemical species. In this way, specific chemical compounds or classes of compounds can be selectively extracted, identified, and quantitated. Also, several aliquots of the sample solution can be passed through the sorbent to achieve preconcentration.

A number of SPE sorbents have been developed and employed in a variety of ways, including concentration and extraction of organic compounds in ground water and surface waters, as was reported in a number of publications and reports (1-3 and

\* Author to whom correspondence should be addressed.



**Figure 1.** Absorption spectra of (A) 5 μM mercury-dithizone complex in 10% acetonitrile-0.05M nitric acid and (B) 5.0 μM dithizone in 10% acetonitrile (pH 4.0).



**Figure 2.** Stabilization of mercury-dithizone complex by immobilization on SPE surface. Plot 1: Complex stability before extraction. Plot 2: Complex stability after extraction and elution from the SPE tube. Plot 3: Complex stability after extraction but left on the SPE tube.

references therein). In other cases, SPE has been successfully applied in conjunction with other analytical techniques in which, for example, SPE provided presample cleanup prior to introduction to gas chromatographic separation (4-8). Several attempts have also been made to modify SPE sorbent surfaces in order to improve efficiency and achieve extraction selectivity (9,10). Multielement species extraction, identification, and quantitation have also been done (11).

Although SPE has clearly demonstrated valuable applications in the study and determination of organic materials in environmental and other media, its use in the determination of metal species has received very little attention. Among the few examples reported include its use in chelation chromatography to remove metal ions from high-ionic-strength media (12), chemical placement of specific functional group ligands on synthetic polymers (13), and a few other cases, including a combination of size and functional group selectivity to achieve specific metal determinations. Some of the SPE materials employed in these examples occur naturally (1,14-17).

Christian et al. (18) used silica and C<sub>18</sub>-bonded silica in combination with graphite furnace atomic absorption to determine trace metals in solution, but no attempts were made to differentiate the different forms of metal that may have been in the sample. A new class of sorption/desorption ion-exchange membrane was developed and characterized for use in trace metal determination in sludges (19). Again, in this case, only total metal was determined, and no attempts were made to look at the different organic and inorganic species that may have been in the sludge materials.

Metal species in the environment can exist in association with organic compounds as neutral or charged complexes. It is therefore important that, when analyzing environmental samples for metal content, the analytical method used has the capability to differentiate these forms from free ions. The work reported in this paper is a preliminary study of the interaction of free and organically bound metal species with SPE materials. The aim was to explore possible applications of SPE in metal speciation. All measurements conducted in the study used synthetic solutions prepared from reagent-grade chemicals.

## Experimental

### Equipment and materials

Two types of SPE tubes were employed, C<sub>18</sub> and LC-NH<sub>2</sub>, both of which were obtained from Supelco (Bellefonte, PA). C<sub>18</sub> SPE tubes consist of reversed bonded-phase packing materials with functionalities similar to those found in high-performance liquid chromatography packing. LC-NH<sub>2</sub> tubes consist of bonded normal-phase packing materials with NH<sub>2</sub> functionality. Solutions of the metals used in this study were prepared by dissolving reagent-grade metal nitrates in distilled deionized water or dilute nitric acid, all of which were atomic absorption grade and were obtained from Fisher Scientific (Fairlawn, NJ). Reagent-grade dithizone and acetonitrile were also obtained from Fisher Scientific.

The extraction of metal species on the solid substrate was

accomplished by connecting the extraction tubes to a vacuum manifold (Supelco), which was operated with a vacuum of about 5 in. of mercury. Flow rates through the extraction tubes were kept constant at about 2.0 mL/min.

Metal–dithizone complexes were measured with a Varian (Mulgrave, Victoria, Australia) model DMS 300 ultraviolet–visible (UV–vis) spectrophotometer. Uncomplexed metal species were measured with a Varian model AA-20 atomic absorption spectrophotometer. Solution pH was measured by using Ion Analyzer, EA 940 (Orion Research, Boston, MA).

Studies with the  $C_{18}$  sorbent were performed using dithizone metal complexes to demonstrate the utility of this device for the extraction of neutral metal complexes. Acetonitrile–water was used as the eluent for the  $C_{18}$  tubes. Studies with LC– $NH_2$  were performed using metal ions in water and acid solutions to demonstrate the utility of this device for the extraction and quantitation of charged metal species. Eluents in this case consisted of plain

water or acid solutions. The sorbents were preconditioned with the appropriate solvent prior to the extraction process.

### Procedure

The extraction of metal species using the SPE method can be done in two ways, depending on the treatment of the sample. If the species of interest is in complexed form, the extraction can be done by direct application of the sample solution on the extraction tube. On the other hand, if the metal species is in free ion form, it will be necessary to form a complex with a suitable ligand first. The complex formed is then applied on the SPE column for extraction. The latter approach was employed in this research.

Metal complexes were made by mixing known concentrations of selected metal ions with excess dithizone. The metal complexes formed and the excess dithizone were applied on the  $C_{18}$  SPE column for extraction. The extraction process was conducted under varying conditions of pH, ionic strength, and time. Extraction studies using the LC– $NH_2$  columns were accomplished by direct application of free metal ions on the column without the formation of complexes. In both sets of study, the aim was to evaluate how the metal species interacted with the solid substrate in terms of their retention characteristics, retention capacity, matrix effects, and elution characteristics and requirements.

## Results and Discussion

### SPE with $C_{18}$ extraction tubes

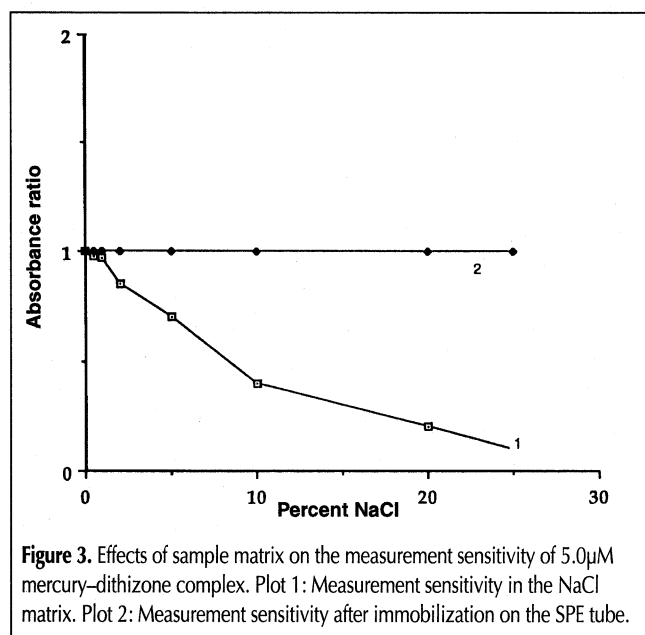
#### Extraction selectivity

Dithizone was chosen for this study as a ligand that forms neutral complexes and to demonstrate the complexity that can be encountered when the extraction process utilizes ligands that not only interact with the extraction material but may also interfere with detection of the extracted metal species. This is demonstrated in Figure 1, which shows the absorption profiles of dithizone and mercury–dithizone ( $5.0\mu M Hg^{2+}$ ). It would be impossible to measure the mercury–dithizone species in the presence of excess dithizone in light of the spectral overlap at 470 nm. To avoid the spectral interference, the two species were selectively eluted. Selective elution was achieved by controlling the acidity of the eluent. Free dithizone was eluted with 10% acetonitrile at pH 4; the mercury–dithizone complex could only be eluted when the acetonitrile was in 0.05M  $HNO_3$ . Under these conditions, over 95% of the metal complex applied on the column was recovered, which was well within the experimental precision of 5%.

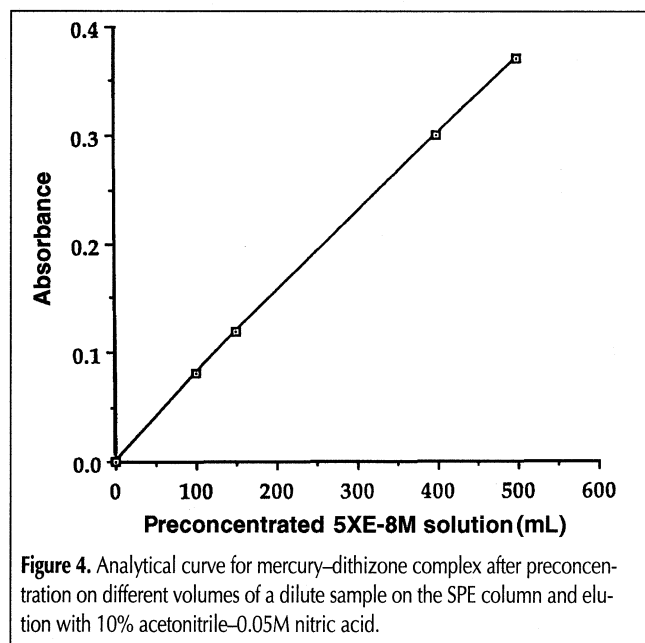
#### Analyte stability

Generally, dithizone decomposes when it is exposed to light. Metal–dithizone complexes are similarly affected by light. However, as depicted in Figure 2, immobilization of the analyte on the solid substrate considerably minimizes analyte decomposition. The plot shows analytical signals obtained for the mercury–dithizone complex under the following conditions.

Plot 1 represents measurements of the unextracted complex left exposed to atmospheric conditions. For plot 2, the complex was extracted and then eluted from the adsorption site; the



**Figure 3.** Effects of sample matrix on the measurement sensitivity of  $5.0\mu M$  mercury–dithizone complex. Plot 1: Measurement sensitivity in the NaCl matrix. Plot 2: Measurement sensitivity after immobilization on the SPE tube.



**Figure 4.** Analytical curve for mercury–dithizone complex after preconcentration on different volumes of a dilute sample on the SPE column and elution with 10% acetonitrile–0.05M nitric acid.

absorbance of the eluted analyte was then measured over a period of time. Plot 3 represents the complex that was extracted, left on the adsorption site, and eluted only immediately prior to absorbance measurements. When the analyte was left immobilized on the solid surface, apparently no degradation occurred. This has important practical applications, especially in field sampling, where it is desirable and necessary to maintain analyte integrity prior to laboratory measurements.

#### Matrix effects

Immobilization of the analyte on the solid substrate isolates it from the sample matrix, which, in addition to preventing it from undergoing photodecomposition as discussed above, separates it from other entities in the sample that might interfere with its detection. This was demonstrated by spectrophotometric measurements of two identical solutions of the mercury–dithizone complex ( $5.0\mu\text{M Hg}^{2+}$ ) in the presence of incremental amounts of sodium chloride. In the first case, aliquots of the mercury–dithi-

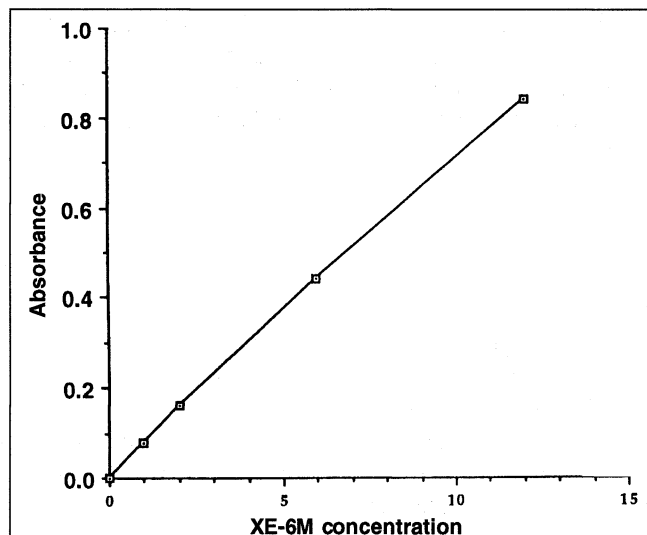


Figure 5. Analytical curve for mercury–dithizone complex after extraction of 5.0 mL of different concentrations and elution with 10% acetonitrile–0.05M nitric acid.

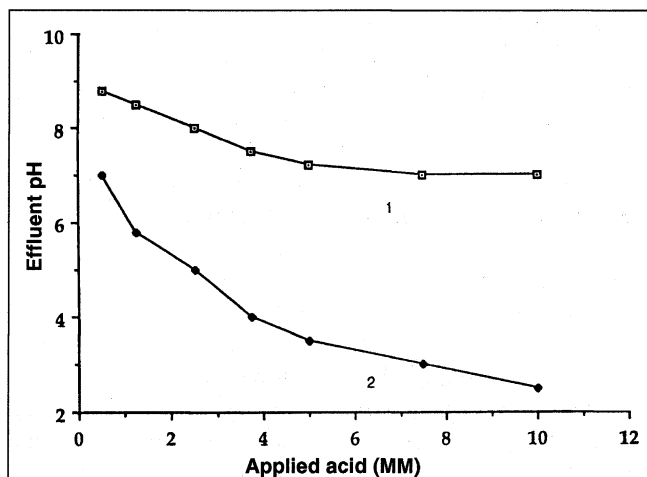


Figure 6. Protonation of LC–NH<sub>2</sub> sorbent by titration with (1) deionized water and (2) 0.01M HCl.

zone complex in different NaCl concentrations were passed through the C<sub>18</sub> tube, followed by their elution with the mobile phase used earlier. The eluted complex was then measured spectrophotometrically. In the second case, amounts of the metal complex with varying NaCl concentrations similar to the first case were measured directly without passing it through the C<sub>18</sub> column. Absorbance ratios of subsequent aliquots were plotted to normalize variations in solvent volumes and composition. As can be seen in Figure 3, when the complex was first immobilized on the solid sorbent, no matrix effects were observed. This is demonstrated by the constant absorbance measured over a wide range of NaCl concentration. When the complex was not extracted from the sample matrix, a progressive decrease in the measured absorbance was observed.

The presence of chloride in the solution interferes with the absorption characteristics of the mercury–dithizone complex in such a way that its molar absorption coefficient at the measurement wavelength of 470 nm is reduced. This interference is eliminated as the integrity of the analyte is restored by removing it from the NaCl matrix.

#### Sample preconcentration

An added advantage to analyte immobilization on solid substrate is that the elution of the extracted analyte uses smaller eluent volumes compared with the sample volume from which it was extracted. This allows sample preconcentration. The requirement, however, is that virtually 100% of the extracted solute is recoverable with a single aliquot of a suitable eluent. The effectiveness of C<sub>18</sub> SPE tubes for preconcentration of metal species was tested by applying varying amounts of a  $5 \times 10^{-8}\text{M}$  solution of the mercury–dithizone complex on the tube, followed by their elution with a fixed 1.0-mL volume of 10% acetonitrile in 0.05M HNO<sub>3</sub>. The effluents were measured spectrophotometrically at 470 nm. Figure 4 shows the linear absorbance–concentration curve that was obtained. Another set of measurements was obtained by applying 5.0-mL volumes of varying concentrations of the mercury–dithizone complex on the extraction tube, followed by elution with 1.0 mL of the acetonitrile–nitric acid

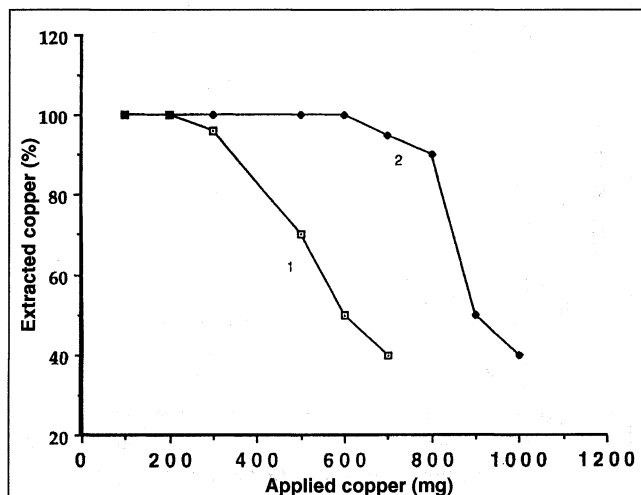


Figure 7. SPE of copper on NH<sub>2</sub> sorbent: Effects of sample acidity in water (1) and in 2.0M HCl (2).

eluent. The corresponding absorbance–concentration curve is shown in Figure 5. Figures 4 and 5 demonstrate the recovery of the extracted solute, which is implied by the linearity of the recovery curves as well as the considerable improvement in measurement sensitivity that can be achieved by extracting the analyte from larger to smaller sample volumes.

#### Extraction with LC–NH<sub>2</sub> SPE tubes

Unlike C<sub>18</sub>, the LC–NH<sub>2</sub> sorbent was not expected to show any significant adsorptive interaction with the neutral metal complexes formed with dithizone. This was found to be the case when the mercury–dithizone complex was applied on an LC–NH<sub>2</sub> column. No retention was observed. Instead, the LC–NH<sub>2</sub> appeared to retain free metal ions, and this retention was highly influenced by the acidity of the solution.

Solution acidity effects were evaluated first by passing deionized water and hydrochloric acid through an LC–NH<sub>2</sub> column and measuring the pH of the solution before and after. As shown in Figure 6, the relatively reactive NH<sub>2</sub> functionality was protonated as the water and hydrochloric acid were passed through the column. The basicity of the site was clearly demonstrated by the raised pH of the applied solutions. As would be expected, the effectiveness of the HCl solution over water in this regard is quite evident.

It is believed that the retention of metal by the LC–NH<sub>2</sub> sorbent occurs through some form of interaction between the metal species and the basic NH<sub>2</sub> group. The medium of the metal species can have a significant influence on the nature and degree of the

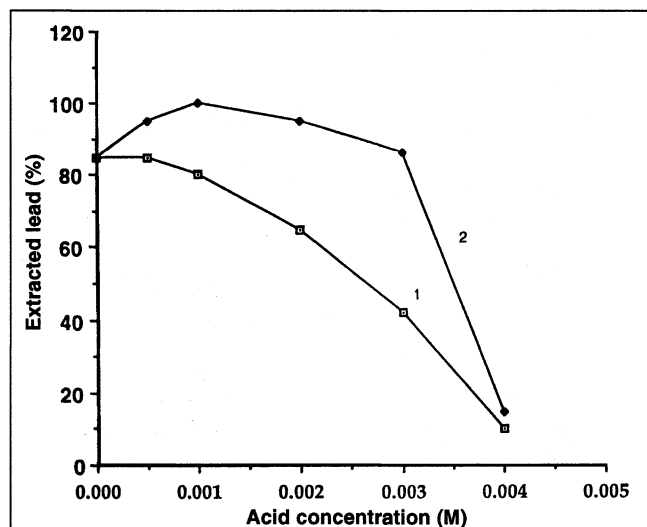


Figure 8. Extraction of copper on LC–NH<sub>2</sub> sorbent after acid preconditioning with (1) HCl and (2) acetic acid.

Table I. Extraction Capacities obtained with LC–NH<sub>2</sub> and Aqueous Solutions

Metal	Extraction capacity (µg/g)
Copper	300
Lead	650
Zinc	300

interaction. If the metal ions are in HCl medium, for example, two processes occur. The NH<sub>2</sub> site becomes protonated and forms NH<sub>3</sub><sup>+</sup>, as demonstrated above; and also, depending on the HCl concentration, metal ions such as Cu<sup>2+</sup> can form negatively charged chloro-complexes of the type Cu(Cl)<sub>4</sub><sup>2-</sup>. These chloro-complexes are then retained on the extraction site by ion association with the NH<sub>3</sub><sup>+</sup>. This is believed to be the nature of the enhanced Cu retention in the 2.0M HCl medium, as depicted in Figure 7. In low HCl concentration or weak acid medium, in which protonation of the NH<sub>2</sub> site is reduced, free metal ions tend to bind directly to the basic NH<sub>2</sub> site, a process which would be more pronounced in acetic acid than in HCl, as depicted in Figure 8.

#### Extraction capacity

Extraction capacities of LC–NH<sub>2</sub> obtained for copper, lead, and zinc are reported in Table I. Extraction capacities were determined by passing aqueous solutions of known concentrations of metal ions through the LC–NH<sub>2</sub> columns and monitoring the effluent for unretained metal. This was repeated until a break-point was reached, which is the point at which 1% of the total amount of metal applied was measured in the recovered solution. As can be seen in Table I, variations do exist from metal to metal.

#### Analyte recovery

Removal or recovery of the immobilized analyte from the extraction site depends on the objective of the extraction process. The process can be for analytical purposes, in which case the extracted analyte would have to be recovered for further measurements and quantitation. The extraction process can also be for purposes of permanently immobilizing the chemical species on the solid surface. In such cases, it would be desirable for the extracted species not to come off but rather to remain immobilized permanently.

Recovery was studied with the C<sub>18</sub> columns only. This was accomplished by applying varying concentrations of metal–dithizone complex followed by elution with 10% acetonitrile–0.05M nitric acid as was done for Figures 4 and 5. Recovery curves similar to the mercury–dithizone curves shown in Figures 4 and 5

Table II. Summary of Recovery Parameters for Several Dithizone–Metal Complexes

Metal complex	Absorbance wavelength (nm)	Extraction coefficient	Concentration range (µM)
Mercury–dithizone	474	2.0 × 10 <sup>5</sup>	0–12
Copper–dithizone	474	2.5 × 10 <sup>5</sup>	0–50
Zinc–dithizone	474	4.0 × 10 <sup>4</sup>	0–40
Cadmium–dithizone	470	2.5 × 10 <sup>4</sup>	0–40
Lead–dithizone	474	2.5 × 10 <sup>4</sup>	0–30

were prepared for lead, cadmium, and copper using the conditions summarized in Table II. Although the concentrations used did not cover a very wide range, it is evident that the extraction process can provide very accurate and reliable results for subsequent analytical measurements or other investigations.

## Conclusion

The work described in this paper shows the analytical applications of C<sub>18</sub> and LC-NH<sub>2</sub> SPE materials in the extraction of metal species. The C<sub>18</sub> lends itself to the extraction of neutral metal complexes, whereas LC-NH<sub>2</sub> is applicable in the extraction of free metal ions in aqueous solutions. In both cases, the extracted metal species can be recovered by employing normal experimental conditions and solvents, allowing further measurements and quantitation.

Both extraction methods can facilitate field sampling, during which the analyte is immobilized on the solid substrate, protecting it from degradation and isolating it from the sample matrix. Sample preconcentration by many factors can also be achieved.

## Acknowledgment

This research was supported by a grant from the U.S. Department of Energy, Division of Chemical Sciences, grant number DE-FG05-86ER13589.

## References

1. K. Terada. Preconcentration of trace elements by sorption. *Anal. Sci.* **7**: 187-98 (1991).
2. H.A. Stuber and J.A. Leenheer. Selective concentration of aromatic bases from water with a resin adsorbent. *Anal. Chem.* **55**: 111-15 (1983).
3. C.E. Rostad, W.E. Pereira, and S.M. Ratcliff. Bonded-phase extraction column isolation of organic compounds in ground water at a hazardous waste site. *Anal. Chem.* **56**: 2856-60 (1984).
4. E. Stottmeister, H. Hermenau, P. Hendel, T. Welsch, and W. Engewald. Solid-phase extraction-programmed temperature vaporizer (PTV) injection in GC analysis of toxaphene and PCBs in aqueous samples. *Fresenius J. Anal. Chem.* **304**: 31-34 (1991).
5. *The Supelco Guide to Solid Phase Extraction*. Supelco, Rohm and Haas, Bellefonte, PA, 1988.
6. P. Dimson. Isolation of phenol and substituted phenols using a cyclohexyl bonded-phase extraction column with HPLC analysis. *LC* **1**: 236-37 (1983).
7. G.C. Yee, D.J. Gmur, and M.S. Kennedy. Liquid chromatographic determination of cyclosporine in serum with use of a rapid extraction procedure. *Clin. Chem.* **28**: 2269-71 (1982).
8. J.J. Vreuls, U.A.T. Brinkman, G.J. de Jong, K. Grob, and A. Artho. On-line solid phase extraction-thermal desorption for introduction of large volumes of aqueous samples into a gas chromatograph. *J. High Res. Chromatogr.* **14**: 455-59 (1991).
9. A.M. Gillespie and S.M. Walters. *Anal. Chim. Acta* **245**: 259-65 (1991).
10. R.S.S. Murthy, L.J. Crane, and C.E. Bronnimann. Characterization of cyano bonded silica phases from solid-phase extraction columns. Correlation of surface chemistry with chromatographic behavior. *J. Chromatogr.* **542**: 205-20 (1991).
11. L. Kadenczki, Z. Arpad, I. Gardi, A. Ambrus, L. Gyorfi, G. Reese, and W. Ebing. Column extraction of residues of several pesticides from fruits and vegetables: A simple multiresidue analysis method. *J. Assoc. Anal. Chem.* **75**: 53-61 (1992).
12. O.J. Challenger, S.J. Hill, P. Jones, and N.W. Barnett. Application of chelating exchange ion chromatography to the determination of trace metals in high ionic strength media. *Anal. Proc.* **29**: 91-93 (1992).
13. J.R. Jezorek and H. Freiser. Metal ion chelation chromatography on silica-immobilized 8-hydroxyquinoline. *Anal. Chem.* **51**: 366-69 (1979).
14. R.A.A. Muzzarelli, F. Tanfani, M. Emanuelli, and S. Gentile. The chelation of cupric ions by chitosan membranes. *J. Appl. Biochem.* **2**: 380-89 (1980).
15. R.A.A. Muzzarelli, R. Rocchetti, and M.G. Muzzarelli. The isolation of cobalt, nickel, and copper from manganese nodules by chelation chromatography on chitosan. *Sep. Sci. Technol.* **13**: 163-73 (1978).
16. R.A.A. Muzzarelli, F. Tanfani, M.G. Muzzarelli, G. Scarpini, and R. Rocchetti. Ligand exchange chromatography of amino acids on copper loaded chitosan. *Sep. Sci. Technol.* **13**: 869-79 (1978).
17. Y. Inaki, M. Otsura, and K. Takemoto. Vinyl polymerization by metal complexes XXXI. Initiation by chitosan-copper (II) complex. *J. Micromol. Sci. Chem.* **12**: 953-70 (1978).
18. R. Atallah, G. Christian, and A. Nevissi. Speciation of parts per billion of metal ions using silica and C-18 bonded silica columns and graphite furnace atomic absorption spectrometry. *Anal. Lett.* **24(8)**: 1483-1502 (1991).
19. S. Sengupta and A.K. Sengupta. Characterization of a new class of sorptive/desorptive ion exchange membranes for decontamination of heavy metals-laden sludge. *Environ. Sci. Technol.* **27**: 2133-40 (1993).

Manuscript accepted May 7, 1997.