



Evaluation of copper speciation in model solutions of humic acid by mini-columns packed with Chelex-100 and new chelating agents: Application to speciation of selected heavy metals in environmental water samples

Jackson K. Kiptoo^{a,*}, J. Catherine Ngila^b, Ned D. Silavwe^c

^a Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya

^b School of Chemistry, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Westville, Durban 4000, South Africa

^c Department of Chemistry, University of Swaziland, P/Bag 4, Kwaluseni, Swaziland

ARTICLE INFO

Article history:

Received 16 April 2009

Received in revised form 28 July 2009

Accepted 28 July 2009

Available online 4 August 2009

Keywords:

Labile metal fraction

Chelating agent

Chelex-100

3-Ferrocenyl-3-hydroxydithioacrylic acid

N,N'-[1,1'-dithiobis(ethylene)]-

bis(salicylideneimine)

ABSTRACT

A solid-phase extraction procedure using mini-columns packed with Chelex-100 and two new chelating agents based on poly(vinyl chloride) functionalized with 3-ferrocenyl-3-hydroxydithioacrylic acid and N,N'-[1,1'-dithiobis(ethylene)]-bis(salicylideneimine) (H₂sales) loaded on microcrystalline naphthalene, is reported. The columns were used to separate labile copper fractions in model solutions and in real samples with subsequent determination using electrothermal atomic absorption spectrometry (ETAAS). Various model solutions containing 20 μg L⁻¹ of Cu²⁺ and 0.0, 0.2, 2.0 and 20.0 mg L⁻¹ of humic acid, respectively, and buffered to pH 6.0, 7.0 and 8.0 were considered. Results showed a decrease in labile copper fraction with increase in humic acid concentration. Application of the procedure to speciation of Cu, Ni, Zn and Pb in various environmental water samples yielded labile fractions in the range of 1.67–55.75% against a total dissolved fraction of 44.08–69.77%. Comparison of the three chelating agents showed that H₂sales had a weaker metal chelating strength than Chelex-100, but PVC-FSSH had comparable chelating strength to Chelex-100.

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1. Introduction

It has long been established that the toxicity, bioavailability, mobility and other critical properties of heavy metals depend on their speciation rather than their total concentrations only [1]. Thus to fully understand the environmental chemistry of an element in a given system, it is important to identify and quantify the various species (forms) that make up its total concentration. Several methods and techniques have been used to determine various heavy metal species and fractions. A recent review by Pesavento et al. [2] details the traditional methods as well as the emerging ones. Of special importance in speciation studies is the distinction between the 'labile' and 'inert' metal fractions. The labile fraction includes the free forms (aqua species, e.g. [Cu(H₂O)₆]²⁺) and simple complexes characterized by fast dissociation rates, while inert fraction represents the strongly bound metal complexes [3]. The labile fraction as an indicator of the bioavailability and toxicity of heavy metals has been supported by both the Free Ion Activity Model (FIAM) [4] and the Biotic Ligand Model (BLM) [5]. For this reason it is useful

to estimate the composition of labile metal forms as a measure of the potential risk posed by heavy metals in environmental water systems.

Chelating resins have found widespread use in heavy metal determination and speciation studies. In particular, the iminodiacetate-based Chelex-100 has been widely used [6–8]. Since the discovery of the ability of Chelex-100 to separate labile metal complexes by Florence and Batley [9], it has been used to determine operationally defined 'labile' and 'inert' metal species in water systems. When the chelating agent is packed in a column, the metal species retained by the column represents the labile forms. Other workers have exploited changes in flow rates to vary contact times and determine 'very labile', 'moderately labile', 'slowly labile' and 'inert' species [10,11]. Although many studies have demonstrated Chelex-100 as an effective chelating agent for solid-phase extraction of labile metal forms in water systems, new chelating agents are being developed to meet emerging ecological, environmental and other research challenges [12–14]. In addition, many studies have not successfully demonstrated the relationship between Chelex-100 separations and indices of biological response such as toxicity and bioavailability [15]. Questions have been raised about the relevance of the type of binding in Chelex-100 columns to the metal binding modes encountered at biomembranes. It has

* Corresponding author. Tel.: +254 067 52181; fax: +254 067 52164.

E-mail address: kiptoojac@yahoo.com (J.K. Kiptoo).

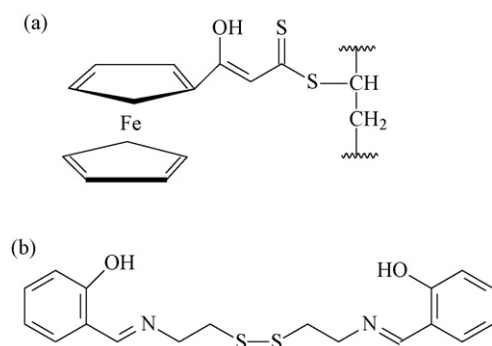


Fig. 1. The structures of poly(vinyl chloride) modified with 3-ferrocenyl-3-hydroxydithioacrylic acid, PVC-FSSH (a) and *N,N'*-[1,1'-dithiobis(ethylene)]-bis(salicylideneimine), *H*₂sales (b).

been suggested that complexation involving thiol groups may be better models since these groups are present in biological systems. For instance, the disulphide group is found in cystine, an important constituent of many proteins as well as lipoic acid, which participates in many enzymatic reactions. This group is well recognized as a potential binding site for metal ions in many biological systems [16].

This paper reports results of studies on new chelating agents based on poly(vinyl chloride) functionalized with 3-ferrocenyl-3-hydroxydithioacrylic acid (PVC-FSSH) (Fig. 1a) and *N,N'*-[1,1'-dithiobis(ethylene)]-bis(salicylideneimine) loaded on microcrystalline naphthalene (Naph-*H*₂sales) (Fig. 1b). Glass mini-columns packed with these chelating agents were used to separate labile metal fractions in model solutions of humic acid and some environmental water samples, with subsequent determination by electrothermal atomic absorption spectrometry (ETAAS). Results are compared with those obtained using the commercially available Chelex-100.

2. Experimental

2.1. Instruments

The determination of metal ions was performed using a Shimadzu AA-6800 electrothermal atomic absorption spectrometer, Shimadzu Corporation (Kyoto, Japan) equipped with a deuterium background corrector, a pyrolytic graphite tube atomizer and hollow cathode lamps. The analytical wavelengths used for zinc, nickel, copper and lead were 213.9, 232.0, 324.8 and 283.3 nm, respectively. Other conditions were similar to those recommended by the manufacturer. A HI 110 Hanna digital pH meter purchased from Hanna Instruments (Póvoa de Varzim, Portugal), was used to measure pH, while a multi-channel Gilson Miniplus 3 peristaltic pump, Gilson International (Viliers Le Bel, France) was used to pump test water samples through the columns. Ultrahigh purity water (UHP) used in this work was obtained by passing distilled water through a Milli-Q reverse osmosis water system, Millipore Corporation (Bedford, MA, USA).

2.2. Reagents

All reagents used were of the highest purity available and were used without further purification. Standard stock solutions (1000 mg L⁻¹) of Zn²⁺, Ni²⁺, Cu²⁺ and Pb²⁺, SAARCHEM (Johannesburg, South Africa) were diluted appropriately with UHP water to obtain the working solutions. Tetrahydrofuran (THF), ethylenediaminetetraacetic acid (EDTA), tris-[hydroxymethyl]aminomethane and naphthalene, Sigma-Aldrich Corporation (St. Louis, MO, USA)

were of analar grade. Other reagents, nitric acid, sodium hydroxide, hydrochloric acid, ammonia and humic acid, Fluka (Butsch, Switzerland) were of spectral purity.

2.3. Preparation of microcrystalline Naph-*H*₂sales

A Naph-*H*₂sales solution was prepared by dissolving 2.0 g of naphthalene and 0.5 g of *H*₂sales in 20.0 mL of tetrahydrofuran. It was then added drop wise into a conical flask containing 200 mL of UHP water with continuous stirring at room temperature. The Naph-*H*₂sales precipitate formed was allowed to stand for 2 h after which it was filtered, washed three times with UHP water and dried at room temperature.

2.4. Preparation of solid-phase extraction (SPE) columns

The SPE columns were prepared by slurry-packing 0.1 g (in UHP water) each of PVC-FSSH, Naph-*H*₂sales and Chelex-100 resin into glass columns of dimensions 50 mm × 2 mm, i.d. Both ends of the column were fitted with small pieces of glass wool (soaked overnight in 10% nitric acid and rinsed several times with UHP water) to retain the resin in the column. A multi-channel Gilson Miniplus 3 peristaltic pump furnished with silicone tubes was used to pump the solutions through the column.

2.5. SPE procedure

The columns were first washed and then preconditioned prior to sample loading. The PVC-FSSH column was washed by passing 10.0 mL of 2.0 M HCl to remove any adsorbed metal. It was then preconditioned with 10.0 mL of 0.1 M Na₂CO₃ at the rate of 1.0 mL min⁻¹, followed by 10.0 mL of UHP water. The Naph-*H*₂sales column was first washed with 10.0 mL of 0.1 M EDTA followed by 10.0 mL of UHP water. The Chelex-100 column was first washed with 10.0 mL of 2.0 M HCl and then converted to the calcium form by passing 10.0 mL of 2.0 M Ca(NO₃)₂ through and finally washed with 10.0 mL of UHP water. Model solutions containing 20.0 μg L⁻¹ of Cu²⁺ and 0.0, 0.2, 2.0 and 20.0 mg L⁻¹ of humic acid were prepared in 0.05 M Tris buffer (tris-[hydroxymethyl]aminomethane). pH was adjusted to 6.0, 7.0 and 8.0 using 2.0 M HNO₃ or 2.0 M NaOH. The model solutions were left to stand for 48 h at room temperature. 50.0 mL of the model solutions were passed through the columns at a flow rate of 0.5 mL min⁻¹. The columns were washed with 10.0 mL of UHP and the adsorbed metal ions were finally eluted with 1.0 mL of 2.0 M HCl (PVC-FSSH and Chelex-100 columns) or 1.0 mL of 0.1 M EDTA (Naph-*H*₂sales column) at a flow rate of 1.0 mL min⁻¹. The eluents were determined for labile copper using ETAAS.

2.6. Speciation of Cu, Zn, Ni and Pb in environmental water samples

The SPE procedure described in Section 2.5 was applied to the speciation of Cu, Ni, Zn and Pb in real water samples, namely surface water, borehole water and brewery wastewater samples. The samples were first filtered through a 0.45-μm membrane filter. Aliquots of the filtered samples (100.0 mL) were then passed through the column at a flow rate of 0.5 mL min⁻¹ and the retained metal was subsequently eluted at 1.0 mL min⁻¹. The eluent was analyzed by ETAAS to obtain labile forms of Cu, Zn, Ni and Pb.

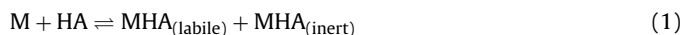
Total metal was obtained by subjecting the unfiltered samples to conventional acid digestion (HNO₃/HClO₄, 3:1) with direct determination by ETAAS. To determine the dissolved metal fraction, the samples were filtered through a 0.45-μm membrane filter and then analyzed directly by ETAAS.

3. Results and discussion

This study compares the ability of three chelating agents with different functionalities to separate labile metal fractions from aqueous solutions. The styrene–divinylbenzene copolymer incorporating iminodiacetate groups (Chelex-100) is well known as a strong chelating agent, and has been used in the equilibrium as well as column techniques in speciation studies. PVC-FSSH (Fig. 1a) was synthesized in our laboratory and has been shown to exhibit strong metal complexing ability through the O,S donor set. It was successfully used to preconcentrate some heavy metal ions from water samples [17]. The ligand *N,N'*-[1,1'-dithiobis(ethylene)]-bis(salicylideneimine) (Fig. 1b) was prepared from the condensation reaction between cystamine and salicylaldehyde following a procedure earlier reported in the literature [16], and incorporated into microcrystalline naphthalene to make it amenable to the column procedure. The disulphide group in *H*₂sales has been shown to exhibit weak metal ligation [16], but is stabilized by the neighbouring ring –OH groups. Our interest in this work was to compare the metal speciation capabilities of the three chelating agents in model solutions as well as in real water samples.

3.1. Effect of humic acid concentration on labile metal fraction in model solutions

Humic substances (including fulvic acids, humic acids and humin) form a major portion of dissolved organic carbon in freshwater systems [18]. Their complexation reactions with metal ions are known to affect trace metal speciation, mobility in the environment and toxicity. Therefore, knowledge of metal binding to humic substances is critical. For this reason, fulvic acids and humic acids are often used to model metal complexation patterns in freshwater systems. We therefore used a commercial humic acid and modeled the pH (6–8) and humic acid concentrations (0.0–20.0 mg L⁻¹) to represent the conditions that obtain in most natural water systems. The Cu²⁺ ion is known to exhibit a strong affinity for natural complexing ligands. For this reason it is often used to model metal complexation reactions in water systems [19–21]. Copper complexation with humic acids has been assumed to be complete within the equilibration period of 48 h. In the separation of labile metal fraction, the competing ligand exchange method (CLEM) is employed with PVC-FSSH, Naph-*H*₂sales and Chelex-100 as competing ligands, with subsequent determination using ETAAS. The following reactions are envisaged: the metal ions interact with the weak and strong binding sites of humic acid to form labile and inert complexes, respectively (Eq. (1)) (charges omitted for simplicity).



In the solid-phase extraction step, the labile metal–humic acid complex is assumed to undergo a first-order or pseudo-first-order dissociation to give the free metal ion, which is then retained by the chelating agent (L) in the column (Eqs. (2a) and (2b)). Thus in principle, the metal fraction retained in the column represents free metal ions and easily dissociated complexes. The CLEM has been applied in similar studies and the theory is well covered in the literature [22].



Recovery values of copper ions extracted by the chelating agents from the model solutions with no humic acid added were quantitative (recoveries >95%) at all pH values for Chelex-100 and PVC-FSSH (Fig. 2a and b), demonstrating the strong chelating ability of the two resins. Using the Naph-*H*₂sales column, quantitative recoveries were obtained at pH 7.0 and 8.0, but not at pH 6.0 (92.43%).

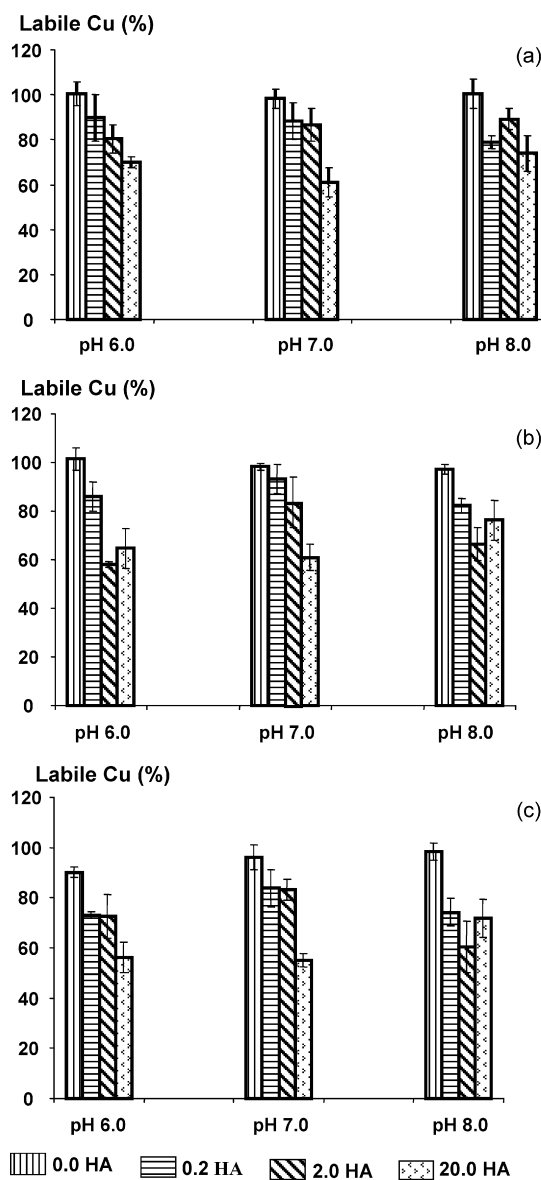


Fig. 2. Labile copper (%) in model solutions humic acid (HA) obtained after sorption onto Chelex-100 (a), PVC-FSSH (b) and Naph-*H*₂sales (c) columns.

*H*₂sales is a Schiff base and is expected to be more sensitive to pH changes. It is possible that *H*₂sales is slightly protonated at this pH, significantly reducing its metal chelating ability. Furthermore, the disulphide group is known to exhibit weak metal chelating property. A significant decrease in the proportion of labile copper fraction with increase in humic acid concentration is observed (Fig. 2a–c). The difference between copper recovery values in the model solutions of copper ions without humic acid (free forms) and those in which humic acid was added, gives the inert forms. Thus the highest composition of inert copper forms are observed in the model solutions with the highest humic acid concentration (20.0 mg L⁻¹).

Humic substances (including humic acids) are irregular polymeric macromolecules with variable structures depending on their origin. Their metal exchange capacity has been attributed mainly to the presence of carboxylic and phenolic hydroxyl groups [23,24]. The complexation capacity of humic acids has been estimated to be about 4.88 mmol g⁻¹ [25]. Based on this, the calculated ratio of binding sites to the metal ion concentration in the model solutions is about 30, 300 and 3000 for concentrations of 0.2, 2.0 and

Table 1
Total, dissolved and labile metal fractions in surface water (SW), borehole (BH) water and brewery wastewater (BW) samples.

Sample identity	Element	Total ($\mu\text{g L}^{-1}$)	Dissolved ($\mu\text{g L}^{-1}$)	Labile fraction ($\mu\text{g L}^{-1}$)		
				Chelex-100	PVC-FSSH	Naph-H ₂ sales
SW	Cu	9.13 ± 2.18	6.37 ± 1.47	4.41 ± 0.58	5.09 ± 0.64	2.45 ± 0.22
	Ni	12.80 ± 1.72	5.77 ± 0.94	2.08 ± 0.66	1.94 ± 0.42	0.88 ± 0.19
	Zn	15.45 ± 2.83	10.16 ± 3.71	5.89 ± 0.42	5.11 ± 0.80	2.14 ± 0.49
	Pb	2.40 ± 0.48	1.06 ± 0.39	0.08 ± 0.02	0.12 ± 0.03	0.04 ± 0.01
BH	Cu	15.01 ± 2.25	8.23 ± 1.96	6.70 ± 1.02	5.32 ± 0.94	3.52 ± 0.32
	Ni	18.06 ± 1.87	7.07 ± 1.85	4.67 ± 0.72	4.02 ± 0.68	2.57 ± 0.44
	Zn	10.12 ± 3.33	8.44 ± 2.15	5.70 ± 1.01	5.17 ± 0.98	3.90 ± 0.89
	Pb	1.93 ± 0.61	0.64 ± 0.04	0.72 ± 0.09	0.85 ± 0.06	0.08 ± 0.02
BW	Cu	24.53 ± 4.31	4.56 ± 1.05	1.09 ± 0.71	0.86 ± 0.09	0.40 ± 0.06
	Ni	16.44 ± 2.47	1.85 ± 0.80	0.43 ± 0.12	0.55 ± 0.08	0.15 ± 0.03
	Zn	42.18 ± 6.14	16.77 ± 2.91	3.39 ± 1.30	3.02 ± 0.89	1.78 ± 0.73
	Pb	7.40 ± 0.99	1.70 ± 0.45	0.54 ± 0.07	0.60 ± 0.07	0.22 ± 0.06

20.0 mg L⁻¹ humic acid solutions, respectively. The labile copper fraction determined using the column technique is assumed to be metal bound to weaker binding sites, and the inert fraction to be bound to strong binding sites. Given that up to a maximum of about 50% of the metal was present as the inert fraction, against a large excess of available binding sites, the implication is that only a small fraction represent strong binding sites.

At low pH, the competition between the proton and the metal ions for binding sites is high. Hence it is expected that lability increases with increase in pH. It should be noted that at high pH, the Chelex-100, PVC-FSSH and Naph-H₂sales are deprotonated and therefore their metal binding strength is expected to increase. Similarly, the carboxyl groups and the phenolic hydroxyl groups in humic acids are also deprotonated at high pH and also expected to bind metal ions strongly. Overall, an increase in pH favours chelating agents owing to their stronger chelating ability, hence the observed increase in lability at higher pH values. In addition, hydrolysis becomes significant at higher pH, further contributing to the labile fraction. This may explain why the labile fraction is generally higher at pH 8.0.

3.2. Speciation of copper, nickel, zinc and lead in environmental water samples

The SPE columns packed with Chelex-100, PVC-FSSH and Naph-H₂sales were also used to separate labile copper, nickel, zinc and lead in real water samples. Surface water (SW) samples were collected from the Okavango delta, Botswana, while borehole (BH) water samples were collected from boreholes around Gaborone city, Botswana. Brewery (BW) wastewater samples were collected from the settling/septic tank of the Kgalagadi Breweries plant situated at Gaborone, Botswana. Table 1 gives the results of total, dissolved and labile Cu, Ni, Zn and Pb. As expected, the values of total metal content obtained after acid digestion was the highest compared to the dissolved and labile metal content. Total metal content comprises of the inert and labile metal complexes as well as particulates. The dissolved fraction obtained by direct ETAAS determination after filtration through 0.45- μm membrane filter were comparable for the surface water (mean 56.94%) and borehole water (mean 52.63%) samples, while it was much lower in the case of brewery wastewater samples (mean 23.14%). This is indicative of the proportion of particulate matter in the samples. Overall, about 50% of the total metal forms are present in the dissolved fraction (11.25–83.40%), while the labile fraction is much lower (1.67–55.75%), depending on the type of column used. The proportion of labile species is dependent on the nature of the competing ligand in the column and the ligands present in the sample. Brewery wastewater had the least proportion of labile species

(1.67–26.83%) followed by freshwater (3.33–45.34%) and borehole water (5–55.75%). This is indicative of the nature of the ligands present in the water system. Brewery wastewater is expected to contain strong complexing ligands, mainly carbohydrates and some protein materials. Thus the metals may be present in strongly complexed forms that cannot be retained in the column. Complexing ability of borehole and freshwater samples may be attributed to humic substances, which are ubiquitous in freshwater systems.

A comparison of the three columns shows that the Naph-H₂sales column yielded the lowest fraction of labile forms (1.67% Pb, 6.88% Ni, 13.85% Zn, and 26.83% Cu). H₂sales bonds through the imine nitrogen, phenolic hydroxo groups and weakly through the disulphide group [16]. Overall, the results show that H₂sales is a weaker chelating agent compared to Chelex-100 and PVC-FSSH. This is consistent with the results obtained when using model solutions of copper.

4. Conclusions

Columns packed with Chelex-100, PVC-FSSH and Naph-H₂sales have been used in speciation studies of copper (20.0 $\mu\text{g L}^{-1}$) in model solutions containing 0.2, 2.0 and 20.0 mg/L of humic acid. Results show that lability decreases with increase in humic acid concentration to a minimum of about 50% at 20.0 mg L⁻¹ of humic acid, suggesting that only a small fraction of the binding sites in humic acids are strong complexing sites. Results from environmental samples (borehole water, river water and brewery wastewater) showed that most of the heavy metals existed in the inert (non-labile) fractions suggesting that strong natural complexing agents are present in these water samples. Comparison between the three chelating agents showed that Chelex-100 and PVC-FSSH were of comparable chelating strengths, while Naph-H₂sales displayed weaker exchange capacity as revealed by the consistently lower values for labile metal fraction in synthetic model solutions and real water samples.

Acknowledgements

The authors are thankful to the German Academic Exchange service (DAAD) for financial support.

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