

## Determination of Zn Species Using Ultrafiltration and Different Solid Sorbents

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### Abstract

A method for the determination of operationally-defined metal species at natural concentration levels has been developed. The method is based on a combination of physical characterization by size fractionation using ultrafiltration and chemical characterization by retention studies on different solid sorbents. The aim of this study is to investigate the effect of chemical and experimental parameters on its performance. A set of three columns packed with different sorbents, namely, a chelating ion exchange resin Chelex-100, an anion exchange resin Dowex 1-X8 and C-18 reversed phase was developed. The retention of metal species in each size fraction onto the sorbents at different pH conditions was investigated. Experiments were performed with metals in the presence of model ligands, namely, nitrilotriacetic acid (NTA), ethylenediamine tetraacetic acid (EDTA), humic acid (HA), 8-hydroxyquinoline-5-sulphonic acid (SOX) and 8-hydroxyquinoline (OX). The applicability of the method was investigated using synthetic river water sample spiked with species of zinc.

*Keywords* : Metal species; ultrafiltration; solid sorbents

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### 1. Introduction

It is known that trace metals such as Cu, Cd, Pb and Zn in natural waters are present in various physical and chemical forms such as free hydrated ions, organic or inorganic complexes and associated with colloidal or suspended particles. The determination of these metals is of increasing interest and important because the impact both their chemical reactivity and biological availability on any environmental systems are strongly depended on the chemical species of metal. The studies of chemical speciation of trace metals are usually based on the discrimination for the operationally defined

classes of metal species having similar chemical properties and reactivities.

The discrimination procedure of metal species is based on the retention of metal forms with different chemical behaviors onto different solid sorbents, namely, chelating ion exchanger, anion exchanger and reversed-phase octadecyl silica. These sorbents have extensively used for the uptake of metal species from aqueous samples and for their enrichment, under different mechanisms of ligands and of complex retention [1].

Liquid-solid extraction is commonly used for the determination of trace metal speciation in natural waters. The most widely used is the chelating ion exchanger Chelex-100 that permits to discriminate

between strongly and weakly bound or free metal species [2]. Many schemes for metal speciation in natural waters based on coupling of retention onto Chelex-100 and spectrometry and electrochemical technique have been developed and reported in several publications. [3-5]. The excellent capacities of strongly basic anion exchange resin for preconcentration and separation of anionic metal complex species are employed by Dowex 1-X8. Yang and Jen (1993) used a Dowex 1-X8 to preconcentration of Cr(III) and Cr(VI) prior to speciation analysis.

Solid phase extraction (SPE) technique is becoming increasing public for speciation analysis of metals in aqueous samples due to the offering for the advantage of high sensitivity of performing a simultaneous enrichment step, and versatility. Its way is used for sample preparation in organometal complexes [7-10].

Among size fractionation methods for speciation studies of trace metals in natural water, ultrafiltration technique is commonly used to separate the dissolved species of metals by passage through molecular filters into various sizes in each molecular level. The size distribution of trace metals in natural waters is determined by meaning of their chemical or physical forms for the evaluation in the impact on any environmental systems.

The objective of this work is to develop a method for the determination of different forms of metal species and to study the effect of chemical and experimental parameters on its performance. The method is based on a combination of physical characterization by size fractionation using ultrafiltration and chemical characterization by retention studies on different solid sorbents. The retention of metal species with different chemical behaviors was studied onto a chelating cation exchange resin Chelex-100, an anion exchange resin Dowex 1-X8 and a reversed-phase  $C_{18}$  sorbent by column method. A set of three columns was packed with the desired sorbents. The behavior of metal species as

free hydrated ions and after conversion into negative or neutral complexes in each size fraction was investigated at different pH values. The ligands employed for the experiments were nitrilotriacetic acid (NTA), ethylenediamine tetraacetic acid (EDTA), humic acid (HA), 8-hydroxyquinoline-5-sulphonic acid (SOX) and 8-hydroxyquinoline (OX). The applicability of the method was investigated by using synthetic river water sample spiked with the species of Zn. The results obtained are discussed in term of percentage distribution of metal species and effect of pH on labilities of desired metal species.

## 2. Experimental

Sample preparation was carried out in a clean room laboratory with the ISSCO laminar flow Model BVT 124 of fume cupboard.

### 2.1 Instrumentation

Metal determinations were performed by using the main two techniques, namely, Differential Pulse Anodic Stripping Voltammetry (DPASV) and Electrothermal Atomic Absorption Spectrometry (ETAAS).

DPASV was performed with Polarography analyzer Potentiostat PGSTAT 100 equipped with Methorm 663 VA Stand using a hanging mercury drop electrode (HMDE), Ag/AgCl reference electrode with salt bridge and Pt counter electrode.

A Perkin-Elmer Zeeman 800 atomic absorption spectrophotometer equipped with HGA-800 graphite furnace, a pyrolytic graphite platform and an AS800 autosampler was used.

Size fractionation of the soluble metal was done by ultrafiltration technique. Ultrafiltration was performed with the "Millipore" Amicon disc membrane filters 76 mm i.d. in a Cole-Parmer stirred cells assembly under  $N_2$  pressure. The NWWL values of membrane filters were 100K, 30K, 10K, 5K and <1K.

The pH was measured by a pH Meter of Denver Instrument Model 15, with a combined glass-calomel electrode of Orion.

## 2.2 Reagents and Materials

The following analytical grade sorbents were used: 100-200 mesh Chelex-100 (Bio-Rad), 100-200 mesh Dowex 1-X8 (Supelco), 37-50 mesh size silica RP-C18 (Sigma). The analytical grade of ligands employed were Na-EDTA (BHD), NTA (Fluka), HA (Fluka), SOX (Fluka) and OX (Fluka). The suprapure grade of acid used as the eluant and an anion exchange resin subjected was HNO<sub>3</sub> (System) and HCl (Merck), respectively. Analytical grade reagents were used: NaOH (Fluka), NH<sub>3</sub> (R&M), CH<sub>3</sub>OH (Merck), Ca(OH)<sub>2</sub> (R&M).

High purity water (HPW) was produced with a Milli-Q system and used throughout.

Standard solution of Zn was obtained by dilution from 1000 mg/L stock solutions (Merck). A working standard concentration of Zn spiked in SRW was 40 µg/L used throughout and prepared fresh as needed.

The synthetic river water (SRW) was prepared by dissolving Ca(OH)<sub>2</sub> and appropriate salts in HPW and giving the pH to 6.5. The analytical concentrations of the principle cations and anions were as follows: Ca<sup>2+</sup> 0.85, Mg<sup>2+</sup> 0.20, Na<sup>+</sup> 0.34, K<sup>+</sup> 0.035, NH<sub>4</sub><sup>+</sup> 0.017; total carbonate 1.7, SO<sub>4</sub><sup>2-</sup> 0.2, Cl<sup>-</sup> 0.38, NO<sub>3</sub><sup>-</sup> 0.008 and total phosphate 0.0030 mM [15]. The concentration of ligands used throughout was as follows: 1x10<sup>-4</sup> M EDTA, 1.6x10<sup>-4</sup> M NTA, 20 µg/L HA, 3 mM SOX and OX. Each of Na-EDTA and NTA was dissolved in HPW. A stock HA was prepared by dissolving 0.100 g of HA in 1L of 0.1 M NaOH, then filtering this solution through a 0.4 µm Nuclepore filter; the residue (~30%) was discarded [16]. OX dissolution was obtained with 0.25 mL of CH<sub>3</sub>OH, then 1.0 mL of 1 M HCl were added and the solution diluted to 200 mL with HPW. SOX was dissolved in a few milliliters of 0.1 M NaOH and the solution brought to volume with HPW. NH<sub>4</sub>COOCH<sub>3</sub> buffer solution

was prepared by mixing the proper amounts of NH<sub>3</sub> and CH<sub>3</sub>COOH to a final concentration of 0.1 M for desired pH used.

All plastic and glassware containers were cleaned by soaking for 48 hr in 10% HNO<sub>3</sub>, then washed with HPW until unretained acid. The stirred cell was cleaned between each experiment series by soaking for 24 hr in a 5% solution of Decon 90 surface active agent.

### 2.2.1 Column parameters and sample reservoir for loading

Glass column, 15 cm. x 1.0 cm. i.d. diameter, containing coarse sintered glass frit and teflon stopcock, at flow rate of 1 mL/min., equipped with 1L. polypropylene separatory funnel with teflon stopcock, joined glass column and separatory funnel with 5.0 cm. polypropylene tubing.

#### 2.2.1.1 Preparation of NH<sub>4</sub>-Chelex column

Weighed 1.3 g of Chelex-100 in the Na-form was allowed to equilibrate in HPW for two days prior to use. The wet resins were then slurried into column, eluted with 10 mL of 2 M HNO<sub>3</sub> to remove trace metals contaminants, and washed with 20 mL of HPW. Then, to convert to the NH<sub>4</sub><sup>+</sup>-form, the resins were subjected to 10 mL of 2 M NH<sub>3</sub> solution, washing with 20 mL of HPW.

#### 2.2.1.2 Preparation of Cl-Dowex column

Dowex 1-X8 3.0 g was washed with HPW until the filtrate became clear. The wet resins were slurry-load into column. The resin had to be activated by passage 30 mL of 1.0 M NaOH through the column, followed by 10 mL of HPW to remove excess NaOH, and then 30 mL of 1.0 M HCl were passed through the column to convert the resin into the Cl form, washing with 10 mL of HPW to remove excess HCl.

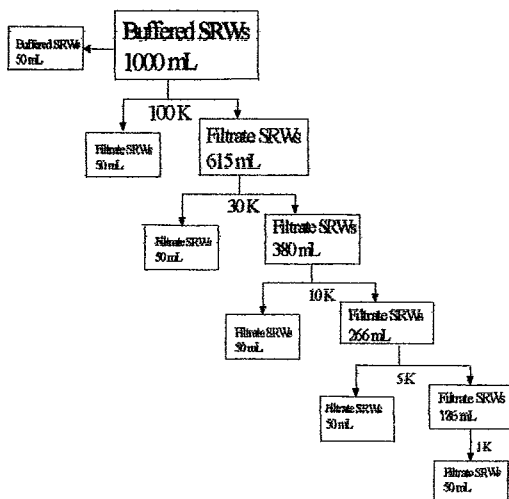
#### 2.2.1.3 Preparation of C-18 column

The silica RP-C18 (1.0 g) were initially cleaned by successive rinsing with 10 mL of CH<sub>3</sub>OH, 10 mL of 1:1 H<sub>2</sub>O : CH<sub>3</sub>OH, 10 mL of 0.6 M HCl and finally 20 mL of HPW.

## 2.3 Procedure

### 2.3.1 Size fractionation by ultrafiltration

The fractionation procedure was begun with the membrane filter having the highest MW cut-off (100 K). The stirred cell and membrane was flushed with HPW. A buffered SRW of 1000 mL was then introduced into the cell and the system pressurized with  $N_2$ . The first 10% and the last 20% of ultrafiltrate were discarded. Only the middle fraction was collected. The ultrafiltrate from the above step was sequentially fractionated using the next lower MW cut-off until that having the smallest cut-off (1K) was used. A flow diagram for size fractionation is shown in Fig. 1



**Figure 1.** Flow diagram for sequential size fractionation by ultrafiltration.

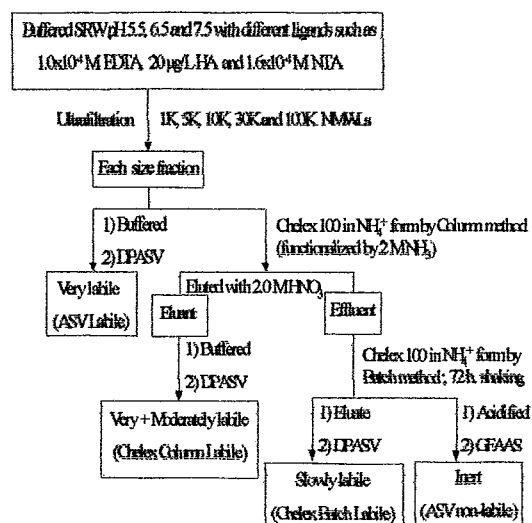
### 2.3.2 Speciation procedure

All ultrafiltrates in each size fraction at different pH values were analyzed in triplicate. Loading and elution flow rates were 1.0 mL/min. The enrichment factor used during optimization was 2.5.

The speciation schemes for the discrimination of the defined classes of metal species in the ultrafiltrate SRW are shown in Fig. 2 to Fig. 4.

### 2.3.2.1 Chelex-100

A portion of ultrafiltrate in each size fraction was determined for "Very Labile" (VLB) fraction before Chelex column passing. The other portions of ultrafiltration in the same size fraction were loaded into the Chelex column for the studies on the degree of lability towards Chelex. VLB and "Moderately Labile" (MLB) fractions were determined from the eluant of Chelex column. "Slowly Labile" (SLB) and "Inert" (INT) fractions were determined from Chelex batch method. All fractions except the INT were measured by ASV whereas the INT fraction was measured by ETAAS.

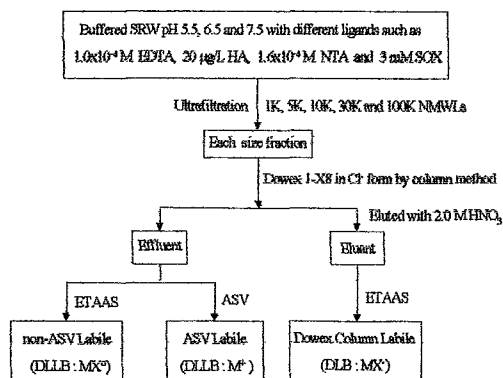


**Figure 2.** Speciation scheme for metal species in SRWs by using Chelex-100.

### 2.3.2.2 Dowex 1-X8

Ultrafiltrate in each size fraction was loaded into the Dowex column for the identification for the defined classes of metal species. Dowex Column Labile (DLB) as negatively charged metal complexes was determined from the eluant of Dowex column by ETAAS. The ASV Labile as a free form of metal species was determined from the effluent of column by ASV. The non-ASV Labile or Dowex Less Labile (DLLB) as neutral form of metal

complexes was also determined from the column effluent by ETAAS.



**Figure 3.** Speciation scheme for metal species in SRWs by using Dowex 1-X8.

### 2.3.2.3 Silica C-18 sorbent

The procedure for the identification of defined classes of metal species by silica C-18 sorbent was similar to Dowex 1-X8 studies. The C-18 Labile (C-18 LB) as neutral metal complexes was determined from the eluant of C-18 column by ETAAS. The ASV Labile as free form of metal species was determined from the effluent of column by ASV. The negatively charged metal complexes as the non-ASV Labile or C-18 Less Labile (C-18 LLB) was determined from effluent of column by ETAAS.

## 3. Results and discussion

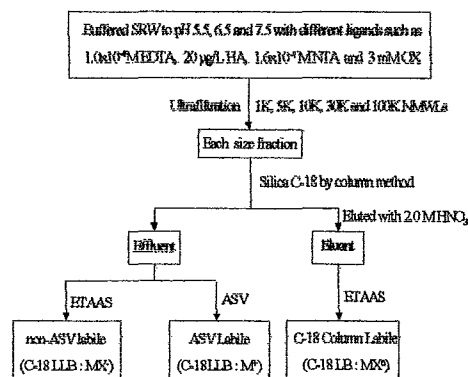
The interaction of three different forms of Zn species onto the different solid sorbents was investigated separately.

### 3.1 Development of method for operational defined classes of Zn species

The species of Zn are categorized into three classification according to its interaction with different kinds of sorbent, i.e. Chelex-100 for positively charge of Zn complexes, Dowex 1-X8 for negatively charge of Zn complexes and reversed phase C-18 for neutral organic of Zn complexes.

Table 1. Operational Definaitons for Metal Classification Scheme onto Sorbent Dependence.

The operational defined classes of Zn species onto different kinds of sorbent are illustrated in the Table 1.



**Figure 4.** Speciation scheme for metal species in SRWs by using silica C-18.

### 3.1.1 Chelex-100

Chelex-100 consists of iminodiacetate groups on styrene-divinylbenzene matrix. The species retained by Chelex-100 are hydrated metal and relatively weak ML complexes. The speciation procedure of Zn in SRW by Chelex-100 is studied according to the suggested method of Figura and McDuffie (1980), that is, the degree of labilities of positively charge of Zn complexes is classified into four types, namely, "Very Labile", "Moderately Labile", "Slowly Labile" and "Inert", as shown in Table 1.

"Very Labile" fraction is the metal species represented as M or hydrated metal ions with fast dissociation kinetics of ML complexes by the approximate time available for dissociation and relative lability to ASV method.

"Moderately Labile" fraction is the metal species that absolutely appeared lability to the Chelex column method and relatively non-lability to the ASV method.

Type of metal classification by different solid sorbents	Labilities of metal species	Measurement technique	Time scale of measurement
Positively charge of metal Complexes by using Chelex-100 uptake	Very Labile (VLB)	Direct ASV	2 ms
	Moderately Labile <sup>a</sup> (MLB)	Chelex column uptake + ASV	7 s
	Slowly Labile (SLB)	Chelex batch uptake + ASV	72 h
	Inert (INT)	Direct ETAAS	—
Negatively charge of metal Complexes by using Dowex 1-X8 uptake	Dowex Labile (DLB)	Dowex column uptake + ETAAS	7 s
	Total Dowex Less Labile (TDLLB)		
	- ASV Labile	Direct ASV	9 s
	- Dowex Less Labile (DLLB)	Direct ETAAS	9 s
Neutral hydrophobic organic metal complexes by using reversed phase C-18 uptake	C-18 Labile (C-18 LB)	Silica C-18 column uptake + ETAAS	7 s
	Total C-18 Less Labile (TC-18 LLB)		
	- ASV Labile	Direct ASV	9 s
	- C-18 Less Labile (C-18 LLB)	Direct ETAAS	9 s

<sup>a</sup>Calculate by subtracting the VLB of metal from the Chelex column results [4].

“*Slowly Labile*” fraction is the non-labile of metal species to the Chelex Column method, but labile by the Chelex Batch method. Such species include relatively stable ML complexes and probably some colloiddally-bound metal.

“*Inert*” fraction is the nonsensitive metal complexes to Chelex Batch uptake as, determined on the effluent from the batch experiment. This fraction represents extremely stable ML complexes, non-labile complexes, or metal adsorbed strongly to colloidal matter.

### 3.1.2 Dowex 1-X8

Dowex 1-X8 is a strongly basic anion-exchanger that is able to obtain anion species in the both acid and alkaline water samples. Although Dowex 1-X8 is suffered from competition of  $\text{Cl}^-$  ion in saline samples, such as seawater, but the utilization of Dowex resin allows a more precise speciation in fresh water than seawater [12]. Therefore the speciation study of anion ML complexes in SRW on Dowex resin is developed. The anion-exchange speciation procedure is based on the charge interaction law, that is, the higher the charge on the ion, the greater is the interaction between the ion and the bed [12]. As already mentioned above, we categorized the degree of labilities of negatively charge ML complexes into three types, namely, "Dowex Labile", "ASV Labile" and "Dowex Less Labile" or "non-ASV Labile" as shown in Table 1.

"Dowex Labile" fraction is the metal species that appeared 100% labile to the Dowex column method and absolute non-labile to the ASV method.

"ASV Labile" fraction is the non-labile of metal species to the Dowex Column method, but relative labile to ASV method. Such species was found in the effluent of Dowex column and represented as a positively charge of ML complexes.

"Dowex Less Labile" or "non-ASV Labile" is the non-sensitive metal complexes to Dowex column uptake, as determined on the effluent from the column experiment. This fraction represents extremely hydrophobic organic ML complexes.

### 3.1.3 Silica C-18

C-18 is a bonded-phase on silica and able to adsorb hydrophobic species and/or partially hydrophilic with a hydrophobic core of ML complexes. The speciation procedure of neutral organic metal complexes by silica C-18 is based on ionic

interaction between ML complexes and the functional group of sorbent. From this reason, the degree of labilities of neutral organic metal complexes was classified into three types, namely, "C-18 Labile", "ASV Labile" and "C-18 Less Labile" or "non-ASV Labile" as illustrated in Table 1.

"C-18 Labile" fraction is the neutral organic metal complexes are retained by reversed phase C-18 column method and entire non-labile to ASV method. Such species include relatively neutral hydrophobic organic ML complexes and partially anion aromatic ML complexes [2, 12].

"ASV Labile" fraction is the non-labile of metal species to the reversed-phase C-18 column method. This species found in the effluent of C-18 column was sensitive to ASV method and typify as a positively charge ML complexes.

"C-18 Less Labile" fraction is the unsusceptible metal complexes to C-18 column uptake, as determined from the effluent from C-18 column. The species found is symbolized as a negatively charge of inorganic ML complexes which are unable to adsorbed on the hydrophilic core of silica C-18.

### 3.2 Determination of Zn species in water

The proposed method was applied to the speciation of SRW and spiked with known amounts of Zn. The percentage recoveries of Zn from each sorbent were evaluated from the ratio of total amount found in the eluates and in the starting concentration in the model solution used. The study of pH effect on retention of Zn-ligands complexes onto different solid sorbents was investigated separately and evaluated at different pH values. The retention of Zn-ligands complexes ones was evaluated at three different pH values, namely 5.5, 6.5 and 7.5 that found in the pH range of natural waters. The results obtained with the proposed method are given in Table 2. to Table 4.





Table 3. Uptake (%) of zinc with different ligands on Dowex 1-X8

pH	Size fraction	SRW			SRW+1.6x10 <sup>-4</sup> M NTA			SRW+1.6x10 <sup>-4</sup> M EDTA			SRW+20 µg/L HA			SRW+ 3 mM SOX		
		Lability (%)			Lability (%)			Lability (%)			Lability (%)			Lability (%)		
		DLB	ASV	non-ASV	DLB	ASV	non-ASV	DLB	ASV	non-ASV	DLB	ASV	non-ASV	DLB	ASV	non-ASV
5.5	<1K	100	-	-	8	90	2	95	-	-	5	50	20	60	-	10
	1K-5K	-	-	-	-	-	-	5	-	-	10	-	-	30	-	-
	5K-10K	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-
	10K-30K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	30K-100K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.5	<1K	99	-	-	12	85	3	94	-	-	4	61	10	87	-	2
	1K-5K	-	-	-	-	-	-	6	-	-	8	-	17	11	-	-
	5K-10K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10K-30K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	30K-100K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5	<1K	99	-	-	18	79	3	92	-	-	2	81	3	93	-	-
	1K-5K	-	-	-	-	-	-	8	-	-	-	-	14	7	-	-
	5K-10K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10K-30K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	30K-100K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

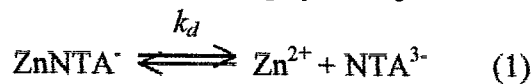
Table 4. Uptake (%) of zinc with different ligands on Silica C-18

pH	Size fraction	SRW			SRW+ 20 µg/L HA			SRW+ 3 mM OX		
		Lability (%)			Lability (%)			Lability (%)		
		C-18 LB	ASV	non-ASV	C-18 LB	ASV	non-ASV	C-18 LB	ASV	non-ASV
5.5	<1K	5	95	-	27	52	-	45	51	5
	1K-5K	-	-	-	8	-	-	-	-	-
	5K-10K	-	-	-	13	-	-	-	-	-
	10K-30K	-	-	-	-	-	-	-	-	-
	30K-100K	-	-	-	-	-	-	-	-	-
6.5	<1K	5	94	-	12	65	-	96	-	-
	1K-5K	-	-	-	24	-	-	4	-	-
	5K-10K	-	-	-	-	-	-	-	-	-
	10K-30K	-	-	-	-	-	-	-	-	-
	30K-100K	-	-	-	-	-	-	-	-	-
7.5	<1K	5	95	-	16	85	-	91	4	-
	1K-5K	-	-	-	-	-	-	4	-	-
	5K-10K	-	-	-	-	-	-	-	-	-
	10K-30K	-	-	-	-	-	-	-	-	-
	30K-100K	-	-	-	-	-	-	-	-	-

a) *Chelex-100*

The experiment was performed by addition of different ligands that simulated in natural waters found, namely, NTA, EDTA and HA, to complex with Zn at three investigated pHs. The results obtained are shown in Table 2. As it can be seen, the complex as  $ZnNTA^-$  occurred when Zn is in the presence of NTA, Zn is mostly retained by Chelex column method and given MLB fraction at the smallest size fraction at all pH values, presumably because the dissociation and protonation of complex occur when increasing pH.

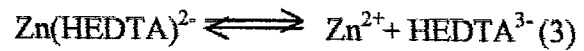
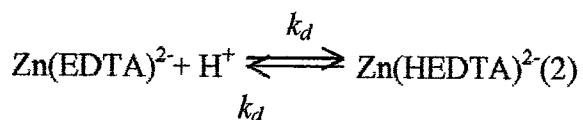
The dissociation of  $ZnNTA^-$  can be represented as occurring by the equation:



where  $NTA^{3-}$  will be absolutely protonated at pH 7.8 and  $k_d$  is a first-order or pseudo-first-order rate constant [11]. The dissociation of complex will be preceded when increase in acidity and given as  $Zn^{2+}$  species. Therefore the retention of Zn decreases when increase pH values. Figura and McDuffie (1979), using Chelex-100 in Ca form, found the same conclusion for Zn-NTA complex in aqueous media.

The complexed  $Zn^{2+}$  in the presence of EDTA at the investigated pH values exists in the form as  $Zn(EDTA)^{2-}$  which is able to protonate and dissociate and releases as  $Zn^{2+}$  by altering the pH. It can be seen that most SLB and partial INT fractions at <1K size fraction are found at all pH values. This retention of Zn is occurred by Chelex batch method that allows for slow dissociation kinetic of metal complexes. It is known that the kinetics of protonation and dissociation of Zn-EDTA complexes at the desired pH values are slowly preceded to release a free form of Zn according to stable complex of  $Zn(EDTA)^{2-}$  [11].

The dissociation of  $Zn(EDTA)^{2-}$  can be illustrated as occurring by the following two reactions:



The protonation step has a pH dependent pseudo-first-order constant,  $k[H^+]$ . The dissociation step will be occurred from limiting supply of protonated species and could not be rate limiting in Chelex batch experiment. According to reaction (2) and (3), dissociation, leading to uptake of Zn by Chelex batch, must be preceded by the protonation step. At increasing pH, this step becomes slower, limiting the supply of  $Zn(HEDTA)^{2-}$  available for dissociation. The low uptake of Zn on the Chelex batch at pH 7.5 is thus in quantitative agreement with this mechanism [11].

According to the complexation of Zn and HA is less effective than NTA and EDTA respectively, thus the formation of Zn-HA complexes is followed by the decrease of the response corresponding to the labile complexed Zn, remaining uncomplexed by HA. It is also presented as  $Zn^{2+}$  [13]. As it can be seen, MLB fraction found as  $Zn^{2+}$  ion is retained by Chelex column at all studied pHs at the smallest size fraction performed whereas Zn-HA complexes are unretained by Chelex column and present as INT fraction at pH 5.5 and 6.5. It is experiment observed in altering the pH that Zn-HA formation is very pH-sensitive as the amount of complexed Zn decrease when increasing pH values. No formation of Zn-HA complexes was found at pH 7.5 that corresponded with the results of Figura and McDuffie (1979).

In the absence of ligand, the absolute species of Zn are VLB fraction and mostly present at <1K of size fraction at all pH values. It is indicating that almost 100% of Zn species presents as aqua ion and partially hydrolyzed forms.

As the mention above, it can be seen that the predominant species of Zn found in the presence ligands to complexation have a relatively increase with increasing pH values, in contrast for Zn in the presence of NTA.

b) *Dowex 1-X8*

The ligands selected to obtain an anion species of Zn were NTA, EDTA, HA and SOX. Table 3. shows the percent retention of Zn with different ligands in different pHs. It is not able to evaluate the retention of Zn species in the absence of ligand whereas the various retentions found of Zn species in the presence of desired ligands are much more interested.

It can be seen that, the uptake of Zn in the presence of EDTA was quantitative and given the absolute DLB fraction as  $Zn(EDTA)^{2-}$  at all studied pH conditions, mostly found in <1K of size fraction. According to the stability constant of  $Zn(EDTA)^{2-}$  complex is 18.3 and it is well known that EDTA forms very stable complexes with most heavy metal so that the presence of negatively charges on the molecule allows their retention onto Dowex resin including with time consuming for complex retention by Dowex column is faster than the kinetic of dissociation and protonation step preceded.

For  $ZnNTA^-$ , it can be seen that partial DLB fractions found are retained by the resin and have relatively increased when increasing pH values. This phenomenon is presumably due to time consuming for complexes retained by the resin and causing the kinetic of dissociation and protonation slowly precede when increase in the pH of sample, as discussed before. On the other hand, as it can be seen, the predominant species ASV labiles found as  $Zn^{2+}$  have a relatively decrease with increasing pH values. The positively charges occurred is accorded to the dissociation of complex. Owing to the stability constant of  $ZnNTA^-$  is 12.0 so that the dissociation of  $ZnNTA^-$  occurs easier than  $Zn(EDTA)^{2-}$ .

The results obtained from complexed Zn in the presence of HA show that some DLB fractions found have a relative decrease with increasing the desired pHs, are not likely in the result of ASV labiles found and nonsignificant difference for amount of non-ASV labiles found; probably as neutral complexes. This phenomenal is

presumably due to the involving interaction in the both of predominant functional groups (carboxylic, phenolic) and hydrophobic component in this molecule, both anion and neutral complexes are found from this experiment. In addition the binding strength will be strongly pH dependent because of the protonation of functional group as phenolic and carboxylic [14], thus the pH altering is effective on amount of the complexed Zn-HA in each form occurred. For ASV labile found, probably due to the efficiency for binding  $Zn^{2+}$  is defective when increasing pH values.

The uptake of SOX complexes onto Dowex was studied owing to simulate the behavior of organic aromatic anion species. The complexes of Zn is quantitatively present as  $Zn(SOX)_2^{2-}$  in all studied conditions, whereas 1:1 and 1:2 (Zn:SOX) complexes exist for Zn at pH 4.0 (from theoretical calculation). The result obtained shows that the retention of Zn at size fraction of <1K increases from 60% at pH 5.5 to 93% at the higher pH. It must be observed that a precipitation of complexes was occurred after elution, due to the formation of barely soluble neutral form of ligand. The precipitate redissolved after 8 h leaving, probably due to the slow protonation of nitrogen and consequent formation of more soluble charge species. It is interesting to note that Dowex 1-X8 also retains the neutral ML forms of Zn. This could be due to the presence of a negative centre located on the sulphonato group, although the overall molecule is uncharged [1].

c) *Silica C-18*

The ligands selected to obtain a neutral complex of Zn species in SRW were HA and OX. Table 4. shows the results obtained of Zn in the presence of desired ligands. It can be seen that most fractions found in the presence of HA are the ASV labile fraction at <1K of size fraction whereas partial C-18 LB at different size fraction and no fraction of non-ASV labile are found. According to silica C-18 is able to adsorb hydrophobic

species or partially hydrophilic ones with hydrophobic core. It is not specific for neutral compounds so that anionic aromatic complexes are retained by the ion exchanger. This phenomenon occurred might be described that the ASV labiles found are unbound HA complex of  $Zn^{2+}$  whereas amount of C-18 LB fractions found is not only neutral organic but also anion Zn-HA complex.

For OX, Zn was partially retained and unbound at pH 5.5 by the meaning of together with neutral complexes, unbound complex as ML and free metal remaining at the conditional pH nearly  $pK_{a1}$  ( $pK_{a1}=4.94$ ). The retention of Zn was completely occurred at pH 6.5 and a slight decrease occurred at pH 7.5. As the experiment retention of Zn in the presence of OX onto silica C-18, it can be probably elucidated that the ratio of Zn:OX complexes are 1:1 and 1:2. The remaining form of complexes is pH dependence, that is, the form presented as  $Zn(OX)^+$  is existed at the lower pH whereas the stable form of complexes is presented as  $Zn(OX)_2$  when the acidity is decreased [2].

#### 4. Conclusions

The developed method present here provides a means for category of soluble metals into degree of lability according to their interaction with different kinds of sorbent. The degree of labilities is classified according to the affinity of their chemical forms for different solid sorbents, i.e., Chelex-100 for cationic species, namely, "Very Labile", "Moderately Labile", "Slowly Labile" and "Inert" fraction, Dowex 1-X8 for anionic species namely, "Dowex Labile", "ASV Labile" and "Dowex Less Labile" or "non-ASV labile" and silica C-18 for neutral organic species, namely, "C-18 Labile", "ASV Labile" and "C-18 Less Labile" or "non-ASV Labile".

This method allows for the speciation study of metals according to a combination

of their physical characterization by size fractionation using ultrafiltration and chemical characterization by retention studies on different solid sorbents. Its applicability to synthetic river water sample spiked with the metal species of zinc in the presence of different ligands at different pH values was demonstrated and discussed by the effect of pH on its retention onto investigated sorbent. The study with model compounds shows that the effect of pH on retention of Zn species onto different kinds of sorbents plays a role to characterize the chemical forms existed, in addition the other interactions occur between Zn and substrates such as some charge hydrophobic species are retained by silica C-18. Anyways the method permits to gain insight into the chemical nature of the metal forms.

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#### References

- [1] Abollino, O., Mentasti, E., Porta, V. and Sarzanini, C., *Anal. Chem.* **1990**, 62, 21.
- [2] Abollino, O., Aceto, M., Sarzanini, C., and Mentasti, E., *Anal. Chim. Acta.* **2000**, 411, 223.
- [3] Batley, G.E. and Florence, T.M., *Anal. Lett.*, **1976**, 9, 379.
- [4] Figura, P. and McDuffie, B., *Anal. Chem.*, **1980**, 52, 1433.
- [5] Chakrabarti, C.L., et al., *Anal. Chim. Acta.* **1993**, 276, 47.
- [6] Ou-Yang, G.L. and Jen, J.F., *Anal. Chim. Acta.* **1993**, 279, 329.
- [7] Lui, Z.S. and Huang, S.D., *Anal. Chim. Acta.* **1993**, 281, 185.

- [8] Tawali. A.B. and Schwedt, G., *Fresenius J Anal. Chem*, **1997**, 357, 50.
- [9] Quináia, S.P. et al. *Talanta*, **2001**, 54, 687.
- [10] Antermidis, A.N., Zachariadis, G.A. and Stratis, J.A., *Talanta*, **2001**, 54, 935.
- [11] Figura, P. and McDuffie, B., *Anal. Chem.*, **1979**, 51, 120.
- [12] Groschner, M and Appriou, P., *Anal. Chem.*, **1994**, 297, 369.
- [13] Kramer, C.J.M. and Duinker, J.C., *Complexation of trace metal in natural waters*. Martinus Nijhoff/Dr W.Junk Publishers, Natherlands, 1984.
- [14] Buffle, J., *Anal.Chim.Acta*. **1980**, 118, 29.