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Claude Fortin<sup>a</sup>; Peter G. C. Campbell<sup>a</sup>

<sup>a</sup> INRS-Eau, Université du Québec, Québec, Canada

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# AN ION-EXCHANGE TECHNIQUE FOR FREE-METAL ION MEASUREMENTS (Cd<sup>2+</sup>, Zn<sup>2+</sup>): APPLICATIONS TO COMPLEX AQUEOUS MEDIA

CLAUDE FORTIN and PETER G.C. CAMPBELL \*

*INRS-Eau, Université du Québec, C.P. 7500, Sainte-Foy, Québec, Canada G1V 4C7*

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A cation ion-exchange technique was calibrated and evaluated in terms of its selectivity for the free-metal ion species in the presence of typical monomeric ligands: chloride, citrate and various amino acids. The method was shown to be highly selective for the free-metal ion in the presence of anionic metal-ligand complexes. For cationic metal-ligand complexes, however, results were more variable: e.g., the Zn-lysine<sup>+</sup> complex interfered with the technique whereas CdCl<sup>+</sup> did not; this contrasting behavior is attributed to the different loci of positive charge in the two complexes. The ion exchange technique was also successfully evaluated for its applicability in complex aqueous media containing natural organic matter (fulvic and humic acids). Adsorption of fulvic or humic acid onto the resin was undetectable and the ion-exchange properties of the resin are unaffected by prolonged pre-treatment with fulvic acid.

*Keywords:* Cadmium; zinc; ion-exchange resin; speciation; fulvic acid; heavy metals

## INTRODUCTION

The presence of toxic metals such as Zn or Cd can jeopardise the quality of aquatic habitats. For a given aquatic organism, biological effects arising from the presence of a particular dissolved metal will normally depend on several environmental variables: e.g., metal concentration, pH and concentration of ligands such as dissolved organic matter<sup>[1]</sup>. These variables can act either directly on the organism (physiological effects) or indirectly by modifying the speciation of the metal in the aquatic medium. According to the free-ion model of metal toxicity<sup>[2,3]</sup>, the biological response of an organism to a metal is proportional to the

\* Corresponding author Fax : +1-418-654-2600. E-mail: campbell@uquebec.ca

free-metal ion activity ( $M^{z+}$ ). Similarly, the geochemical behavior of a metal in the aquatic environment (adsorption / desorption; precipitation / dissolution) is also predictable on the basis of the free-metal ion concentration. Given these key roles of the free-metal ion in natural waters (from the geochemical as well as the toxicological perspective), it is obviously important to be able to quantify free-metal ion concentrations in diverse aquatic matrices. In defined media, metal speciation can usually be calculated from first principles (given the solution composition and the appropriate stability constants for the various metal-ligand complexes, ML). However, this approach will not work in the presence of natural dissolved organic matter (DOM: humic and fulvic acids) for which we do not have the appropriate thermodynamic complexation constants. It follows then that metal speciation must be measured experimentally in such systems.

Cantwell *et al.*<sup>[4]</sup> developed a column equilibration ion-exchange technique (IET) using a cation exchange resin to measure free  $Ni^{2+}$  concentrations in waste waters. The method was tested with model ligands such as EDTA and glycyl-L-alanine. Treit *et al.*<sup>[5]</sup> then miniaturised the method and calibrated it for free cupric ion measurement. This same setup was further tested by Sweileh *et al.*<sup>[6]</sup>, who compared ion-exchange and ion-selective electrode (ISE) measurements of free  $Cu^{2+}$  with calculated values for several Cu-ligand systems (phthalate, glycine, citrate, chloride, salicylate and fulvic acid). In the presence of citrate and fulvic acid, results were identical for both the ISE and the IET. However, concentrations of  $Cu^{2+}$  obtained with the IET were significantly higher than those estimated potentiometrically when phthalate, glycine or salicylate were used as ligands, suggesting interferences from neutral and/or cationic species. ISE measurements could not be performed in the presence of chloride but IET values were consistent with those obtained from equilibrium speciation calculations.

Werner<sup>[7]</sup> calibrated a batch method using a cation exchange resin (with Ca and Mg as counter-ions) to determine free  $Cd^{2+}$  and  $Zn^{2+}$  concentrations in solution. Experiments with NTA showed a good correlation between measured and calculated concentrations of free-metal ions. Other batch methods have been used to determine free  $Cd^{2+}$  ion concentrations. Results from Holm *et al.*<sup>[8]</sup> and Desnoyers<sup>[9]</sup> indicate a good selectivity for  $Cd^{2+}$  in the presence of chloride and sulfate, two ligands which tend to form neutral and/or cationic complexes.

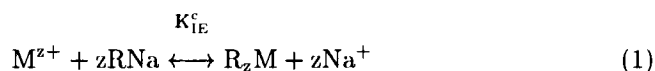
More recently, Ren and Kratochvil<sup>[10]</sup> have modified the IET to measure  $Ca^{2+}$  and  $Mg^{2+}$  concentrations in urine samples. This time, the method was calibrated for varying concentrations of major cations (Na, K, Ca and Mg). The method was tested with citrate, for which thermodynamic formation constants are well known for all the relevant metal-citrate complexes. In a second study,

the IET was used to determine an equilibrium constant for the complexation of Mg with malonic acid, using potassium as a counter-ion<sup>[11]</sup>.

In the present study we have tested the IET in the presence of ligands forming both cationic and anionic complexes and have compared measured and calculated free-metal ion concentrations, with particular emphasis on those conditions where positively-charged complexes are present in solution. Standard fulvic and humic acids have been used to evaluate the capacity of the IET to measure the free-metal ion concentration in the presence of natural dissolved organic matter.

## THEORY OF METHOD

In the presence of an ion-exchange resin (in the sodium form), an equilibrium can be established with a trace metal  $M^{z+}$  (1). This reaction can be described by the equilibrium constant  $K_{IE}^c$  (2),



$$K_{IE}^c = \frac{[R_zM][Na^+]^z}{[M^{z+}][RNa]^z} \quad (2)$$

where R = resin, RNa = resin binding sites occupied by  $Na^+$ ,  $R_zM$  = resin binding sites occupied by metal  $M^{z+}$ , and  $z$  = number of binding sites involved in retaining metal M. In the presence of a sufficient amount of strong electrolyte, such as  $NaNO_3$ , concentrations of sodium in solution,  $[Na^+]$ , and on the resin,  $[RNa]$ , will not be significantly affected by the exchange of metal ions. Trace conditions are fulfilled when the metal M occupies less than 1% of the total resin sites ( $[RNa^+] \gg [R_zM]$ ). At fixed ionic strength and pH, equation (2) can be rearranged to yield a distribution coefficient  $\lambda_{o,i,pH}$  (expressed in  $L \cdot g^{-1}$ ):

$$\lambda_{o,i,pH} = K_{IE}^c \frac{[RNa]^z}{[Na^+]^z} = \frac{[R_zM]}{[M^{z+}]} \quad (3)$$

The metal bound to the resin ( $R_zM$ ) can be measured experimentally by eluting the resin with a volume  $V$  of strong acid. According to the quantity of resin used ( $m_r$ ) and the concentration of metal measured in the eluate,  $[R_zM]$  can be calculated by equation (4):

$$[R_zM] = \frac{[M_{Eluate}] * V}{m_r} \quad (4)$$

Combining and rearranging equations (3) and (4) gives the relationship between the concentration of metal bound to the resin and the free-metal ion concentration in solution:

$$[M^{z+}] = \frac{[M_{\text{Eluate}}] * V}{\lambda_{o,i,pH} * m_r} \quad (5)$$

Once a distribution coefficient specific to the metal of interest has been determined, for the relevant concentration and nature of the electrolyte and for the pH of the solution, one can easily calculate  $[M^{z+}]$ .

## MATERIALS AND METHODS

### Reagents and solutions

All plasticware used was made of high density polyethylene (HDPE), polypropylene copolymer (PPCO) or tetrafluoroethylene (TFE). These containers and other equipment used were acid-washed (10% HNO<sub>3</sub>) for at least 24 hours, rinsed thoroughly seven times with ultrapure water (resistivity ~18 Mohms·cm<sup>-1</sup>) and dried under a laminar flow hood. All manipulations were performed under the laminar flow hood, wearing powder-free gloves, to minimize Zn contamination. Stock solutions of metals were prepared from atomic absorption standards. Analytical grade reagents or better were used for nitric acid (JT Baker Trace Metals grade or BDH Aristar), sodium hydroxide (BDH Aristar or Alfa Ultrapure), EDTA (Fisher Scientific), citrate (BDH), sodium chloride (Merck suprapur), alanine (SigmaUltra >99%), cysteine (Sigma >98%) and lysine (Sigma free base >98%). Laurentian fulvic and humic acids were obtained from Eco-Linc (Roxboro, Canada) and used as supplied. Sodium nitrate (BDH AnalaR) was further purified using a Chelex 100 resin (Bio-rad) according to the following procedure<sup>[12]</sup>: 40 g of Chelex in the sodium form was added to 1 L of a 2.0 M NaNO<sub>3</sub> stock solution and stirred gently for 48 hours at a pH of 7. The suspension was filtered (Millipore, mixed cellulose acetate and nitrate, 0.45 μm) and the filtrate stored at room temperature.

The analytical grade cationic resin (sulfonic acid functional groups) was purchased from Sigma (Dowex 50W-X8, 50–100 mesh). Measurements of pH were made with a Fisher Accumet 915 pH-meter, using an AccupHast glass combination electrode. Concentrations of Zn and Cd were determined using a Varian SpectrAA 20 or 300 (flame or flameless) atomic absorption spectrophotometer; calibration was performed with standard solutions prepared in analytical matrices matched to the samples to be analyzed.

## Apparatus and procedure

Two experimental setups were used, one using large columns and the other being a miniaturised version. Both methods involve passing a sufficient amount of the solution to be analyzed, containing 0.20 M  $\text{NaNO}_3$  as the swamping electrolyte, through the column so as to reach a steady state, i.e. the metal concentration of the solution coming out of the column is equal to the initial concentration ( $[\text{M}^{z+}]_{\text{out}} = [\text{M}^{z+}]_{\text{in}}$ ). The two procedures are thus equivalent, the differences residing in the amount of resin, the size of the column, the amount of solution and the time necessary to process one sample.

## Manual IET

The first method (referred to hereafter as the manual IET) is described in detail by Cantwell *et al.*<sup>[4]</sup>. Preliminary experiments (data not shown) indicated that 500 mL of a  $\text{NaNO}_3$  solution (0.20 M) containing Cd or Zn was sufficient to reach a steady-state equilibrium between the resin (300 mg dry weight) and the solution at a flow rate of  $5.5 \pm 0.5 \text{ mL}\cdot\text{min}^{-1}$ . Also, 50 mL of the acid solution ( $\text{HNO}_3$ , 1.5 M) was necessary to elute all the metal from the resin after equilibrium. The resin was placed in a glass column (30 cm long; 1 cm internal diameter) and supported on a porous polyethylene frit. Control experiments performed without any resin indicated no significant metal retention by adsorption on the column surface. Resin purification and its conversion to the sodium form were performed as described by Cantwell *et al.*<sup>[4]</sup>.

The manual IET procedure involved the following steps. First, 300 mg of resin ( $m_r$ ) in the sodium form was inserted into the column with a 0.20 M  $\text{NaNO}_3$  solution (without metals) at a specific pH. The supporting electrolyte solution was passed through the column until the pH had stabilised to the set level. Second, 500 mL of the metal solution (with 0.20 M  $\text{NaNO}_3$ ) was passed through the resin at a flow rate of  $5.5 \pm 0.5 \text{ mL}\cdot\text{min}^{-1}$ . The pH was monitored at least three times during this step. Third, when all the solution had left the column, 10 mL of ultrapure water was passed through the column to remove the remaining interstitial solution. A slight air pressure was applied to force the liquid out of the column. Finally, a volume ( $V$ ) of 50 mL of  $\text{HNO}_3$  1.5 M was passed through the column to recover the metal ( $[\text{M}_{\text{eluate}}]$ ) from the resin. Pressurised air was again used to evacuate all the acid. Samples were collected in HDPE containers and stored in a refrigerator until analysis.

## Miniaturised IET

The manual IET requires large amounts of solution and considerable preparation time. We explored the possibility of miniaturising the method, using as a starting

point the earlier work by Treit *et al.*<sup>[5]</sup> and by Sweileh *et al.*<sup>[6]</sup>. These latter authors had connected the IET column directly to a flame atomic absorption spectrophotometer (AAS). This approach has several disadvantages: high detection limits, tedious daily calibrations, time variations in the absorption signal, monopolisation of the AAS instrument. The modifications that we incorporated are similar to those described recently by Ren and Kratochvil<sup>[10]</sup>, but were undertaken independently.

A diagram of the setup is shown in Figure 1. Solutions were pumped through a column containing a precisely weighed amount of the Dowex 50W-X8 resin (between 7.6 and 7.8 mg) by simply selecting the proper channel with a 6-way rotating Teflon valve (Milton Roy). All tubing and connections were made of Teflon except for the section going through the pump head (Technicon green-green Tygon tube). Nitric acid was pipetted into a Teflon funnel (Varian) for the elution step; this manual step ensured that the volume added was known with high accuracy, this being crucial for the calculation of the free-metal ion concentration (eq. 5). «On-line» pH monitoring of the solution coming out of the column was possible by using a thin neck pH electrode and a small 2 mL vial so that the flushing rate of solution was fast. A 20 × 20 × 20 cm cubic acrylic box was used as a physical support for the solutions, tubing, funnel and the rotating valve.

Preliminary experiments showed that 20 mL of solution was sufficient to obtain a steady-state response from the resin column and that 2 mL of HNO<sub>3</sub> (1.5 M) was enough to elute the resin at a reduced flow rate (0.5 mL·min<sup>-1</sup>, to maximise contact time). The following sequence was observed for all measurements (flow rate = 5.0 mL·min<sup>-1</sup> unless otherwise noted): i) Conversion of the resin to the sodium form by pumping a NaOH (0.10 M) solution for two minutes; ii) Washing with ultrapure water for four minutes; iii) Adjustment of resin pH with NaNO<sub>3</sub> (0.20 M) for approximately two minutes (until pH was stable at the desired level); iv) Column-equilibration with the test solution for four minutes (20 mL); v) Washing with ultrapure water for five seconds; vi) Elution of the resin (flow rate = 0.5 mL·min<sup>-1</sup>) with 2.0 mL of nitric acid (1.5 M) and recovery of the eluate in a 4 mL HDPE container; vii) Washing with ultrapure water for four minutes. Since the volume of elution is included in the calculation of the free-metal ion concentration, all containers used to recover the eluate were weighed before and after elution to account for any losses of acid in the tubing. The eluate was then analysed by atomic absorption spectrophotometry for total metal concentration. Low concentrations of pH buffers were used occasionally to control solution pH (potassium phthalate 0.10 mM for pH 5.5 and TRIS 0.50 mM for pH 7.0).

The success of the miniaturised IET depends to a large extent on the ability to prepare the miniature resin columns in a reproducible manner. We have used a different and simpler approach than the one published by Treit *et al.*<sup>[5]</sup>. Using heat-sensitive TFE tubes (Cole-Parmer 06851-10) that contract from 1.93 to 1.25 cm diameter upon heating above 327°C, the resin was loosely trapped between two porous glass frits (1.5 mm diameter, ~2 mm length and 50 µm porosity). First, one frit was inserted into the tube and its area was carefully heated until the frit was tightly secured by the shrunk tube. When the tube had cooled to room temperature, the precisely weighed resin was suspended in ultrapure water and inserted into the column with a micropipette. A second frit was then placed at the top of the column and heat-sealed into position. The bottom section of the column was carefully wrapped with aluminium foil and shielded with glass to protect the resin from the heat source (the resin can be altered at temperatures > 150°C). Only the top area of the column was heated to secure the second frit. Finally, the two tips of the column were heated to join the column to the Tygon tubing, connected to the pump. The details of the column are shown in Figure 1 (inset).

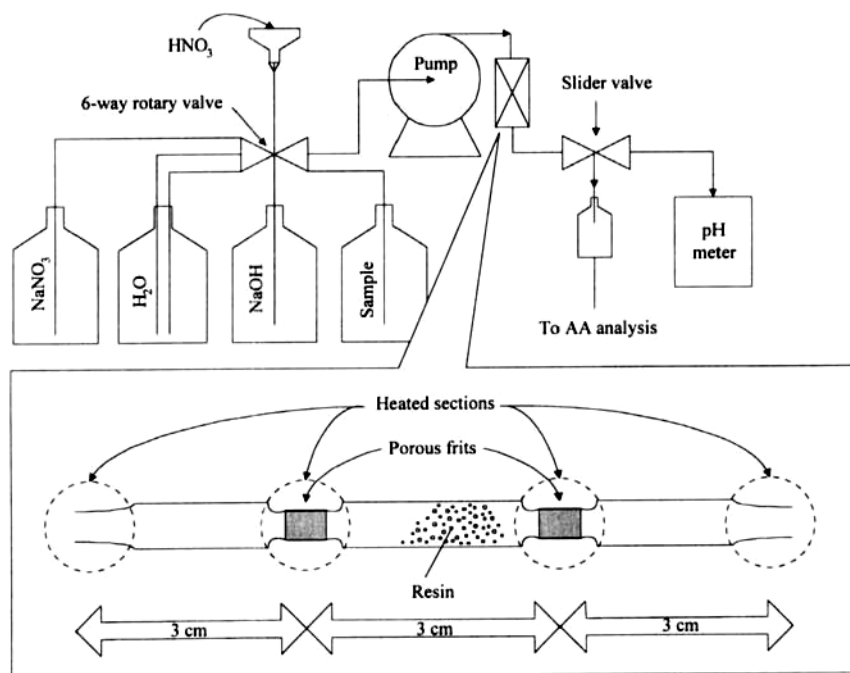


FIGURE 1 Experimental setup for the miniaturised ion exchange technique, showing details of the column construction



### Thermodynamic calculations

Metal speciation at equilibrium in the various solutions was calculated with the computer programme MINEQL+<sup>[13]</sup> and updated thermodynamic formation constants from Smith *et al.*<sup>[14]</sup>. These results were compared with the values obtained with the IET. Particular attention was given to the choice of the formation constants. The default thermodynamic database for the MINEQL+ / MINTEQA chemical speciation programmes contains numerous errors<sup>[15-17]</sup>. Priority was therefore given to values in the NIST Standard Reference Database<sup>[14]</sup>. These data were compiled in the *personal.dat* file, which can be uploaded in MINEQL+ [available at our <http://www.inrs-eau.quebec.ca/activites/groupe/biogeopersonal.htm>].

MINEQL+ is inadequate for calculating metal speciation in the presence of fulvic and humic acids. For such solutions, we used the WHAM equilibrium model recently developed by Tipping<sup>[18]</sup> and tested model predictions against values of Cd<sup>2+</sup> and Zn<sup>2+</sup> measured in the presence of Laurentian fulvic acid (Ecolinc).

### Calibration

Distribution coefficients were evaluated for both Cd and Zn at several pH values. To evaluate these coefficients using equation (5), the free-metal ion concentration of standard solutions containing no ligands must be known. Although thermodynamic formation constants for Cd and Zn complexes with nitrates are low, at the high NO<sub>3</sub><sup>-</sup> concentration used (0.20 M) these complexes become significant. Thus, free-metal ion concentrations, [M<sup>2+</sup>], in these calibration solutions were also calculated using MINEQL+.

### DOM-resin interactions

Experiments with humic substances were conducted to test for possible adsorption of DOM onto the resin. The first type of experiment consisted of exposing variable amounts of resin to a known concentration of fulvic acid (FA) or humic acid (HA) and monitoring this concentration. A decrease in FA or HA concentration would indicate adsorption onto the resin. Since hydrophobic interactions with humic substances are likely to occur at low pH<sup>[19]</sup>, the lowest pH for which the IET was calibrated (4.5) was chosen for these interference tests. Using a UV-visible spectrophotometer ( $\lambda = 400 \text{ nm}$ <sup>[20]</sup>, 5.5 mL cells, Varian DMS 200), we obtained a calibration curve (data not shown) for standard solutions of fulvic acid over the concentration range from 7.0 to 11.0 mg·L<sup>-1</sup>. In batch experiments, Laurentian fulvic acid solutions (10 mg·L<sup>-1</sup>; 100 mL) were placed in 125 mL

flasks containing 0, 0.5, 1.0 and 2.0 g of resin, with three replicates for each concentration. As a control, three additional flasks contained 2.0 g of resin with 100 mL of ultrapure water (no FA). After eight hours, each solution was filtered (Millipore, mixed cellulose acetate and nitrate, 0.45  $\mu\text{m}$ ) and re-analysed by UV-visible spectrophotometry. Three readings were taken for each replicate. Using the same methodology, another experiment was performed with 16  $\text{mg}\cdot\text{L}^{-1}$  of Laurentian humic acid with both 0 and 20  $\text{g}\cdot\text{L}^{-1}$  of resin.

### Experimental plan

Three groups of experiments were carried out: i) Tests with model ligands forming anionic complexes for which reliable thermodynamic data are available (EDTA and citrate); ii) Experiments to test the selectivity of the resin towards the free-metal ionic species,  $\text{M}^{2+}$ , in the presence of ligands that form neutral or cationic complexes with Cd or Zn (chloride, alanine, cysteine and lysine); iii) Experiments with natural dissolved organic matter (Laurentian fulvic acid) to determine free-metal ion concentrations. The details of these individual experiments are compiled in Table I.

TABLE I Summary of experiments performed to test the IET

<i>Ligand name</i>	<i>[Ligand]</i>	<i>[Cd] (nM)</i>	<i>[Zn] (nM)</i>	<i>pH</i>
EDTA	300 – 720 nM	89	760	5.5
Citrate	0.04 – 4 mM	89	150	5.5
Chloride	3.5 – 200 mM	89	ND	5.5
Alanine	5 – 100 mM	180	ND	7.4
Cysteine	0.05 / 50 mM	ND	230 – 780	6.9 / 4.5
Lysine	10 – 100 mM	ND	760	7.0
Fulvic acid	10 $\text{mg}\cdot\text{L}^{-1}$	4.4 – 320	76 – 610	5.5

## RESULTS AND DISCUSSION

### Calibration

In the calibration experiments, free-metal ion concentrations varied between 3.7 nM and 1.8  $\mu\text{M}$ , through which range the distribution coefficients were constant

(Table II). As expected, the distribution coefficients increased with pH and were greater for Cd than for Zn, reflecting the known higher specific affinity of the resin functional groups for Cd. The slight differences between the distribution coefficients determined for the manual and the miniaturised methods are possibly related to the difference in Na conversion. The average normalised concentration of sodium on the resin was higher for the miniaturised method ( $4.4 \pm 0.1$  meq·g<sup>-1</sup>, n=7) than for the manual method ( $3.7 \pm 0.3$  meq·g<sup>-1</sup>, n=5) for the same pH (5.5), suggesting that the resin was less efficiently converted to the sodium form in the manual method. However, distribution coefficients are operationally defined and the slight differences between the manual and miniaturised methods do not detract from the IET as long as calibration and measurements are made under the same conditions and with the same methodology.

TABLE II Distribution coefficients for the IET

Method	pH	$\lambda_o^{Zn} \pm S.D. (L \cdot g^{-1})$	$\lambda_o^{Cd} \pm S.D. (L \cdot g^{-1})$	n
Manual	4.5	0.38 ± 0.04	0.50 ± 0.01	16
Manual	5.5	0.41 ± 0.03	0.52 ± 0.02	28
Manual	7.5	ND	0.58 ± 0.02	8
Miniaturised	4.5	0.32 ± 0.03	0.36 ± 0.02	9
Miniaturised	5.5	0.39 ± 0.02	0.44 ± 0.02	9
Miniaturised	7.0	0.63 ± 0.07	0.70 ± 0.06	9

The sensitivity of the IET is affected by the instrument used to determine the metal concentration in the column eluates. The detection limit of the atomic absorption spectrophotometer was estimated from the analytical precision of the determination of the Zn and Cd concentrations in a typical eluate (n=10). The lowest detectable concentrations, defined as 3x the standard deviations of these measurements, were 0.2 and 0.5 nM for Cd<sup>2+</sup> and Zn<sup>2+</sup> respectively. From these values, the sensitivity of the IET was evaluated by calculating the variability in free metal-ion concentrations caused by the statistical uncertainty of all the distribution coefficients determined (Table II). Using equation (5), substituting the AAS detection limit for [M<sub>Eluate</sub>], and using  $\lambda_{o,i,pH} \pm$  three times the standard deviations of  $\lambda_{o,i,pH}$  for both Cd and Zn, we obtained concentration sensitivity ranges of 0.06–0.2 nM Cd<sup>2+</sup> and 0.3–0.6 nM Zn<sup>2+</sup> respectively, depending on pH and method (manual or miniaturised).

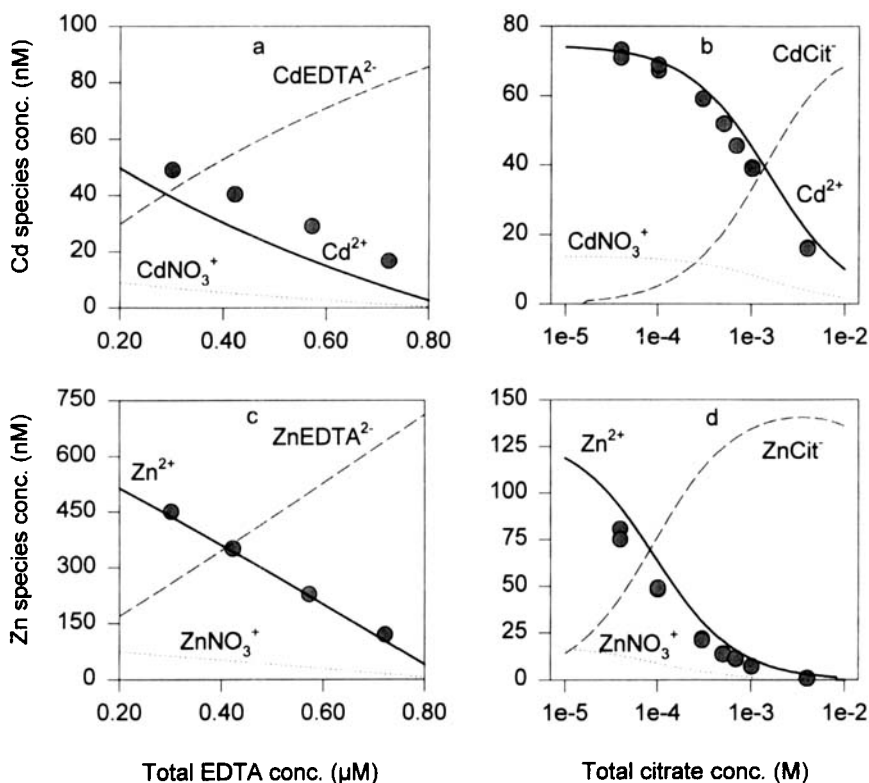


FIGURE 2 Comparison of calculated and measured concentrations of  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  in the presence of EDTA and citrate at pH 5.5. a) Cd-EDTA,  $[\text{Cd}]_{\text{tot.}} = 89 \text{ nM}$ ; b) Cd-Citrate,  $[\text{Cd}]_{\text{tot.}} = 89 \text{ nM}$ ; c) Zn-EDTA,  $[\text{Zn}]_{\text{tot.}} = 765 \text{ nM}$ ; d) Zn-Citrate,  $[\text{Zn}]_{\text{tot.}} = 150 \text{ nM}$ ; ●: Free-metal ion concentration measured with the IET; Lines represent concentrations of major species calculated with MINEQL+

### Resin selectivity – model ligands forming anionic complexes

Tests with EDTA and citrate, two model ligands forming anionic M-L complexes, showed good selectivity for the free-metal ion. Figures 2a and 2c show how theoretical and measured values of  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  compare in the presence of EDTA ( $[\text{EDTA}] = 300, 420, 570 \text{ and } 720 \text{ nM}$ ). Although measured concentrations of  $\text{Cd}^{2+}$  were somewhat higher than the predicted values, the agreement was considered satisfactory, whereas for  $\text{Zn}^{2+}$  agreement was excellent. A wide range of citrate concentrations was used for the second experiment, from 0.040 to 4.0 mM. Experiments with Cd and Zn were performed independently with two replicates for each citrate concentration. Free-ion measurements are illustrated in

Figures 2b and 2d. The  $\text{Zn}^{2+}$  results were slightly lower than the calculated values (Figure 2d) whereas for  $\text{Cd}^{2+}$  measured and calculated values were in very good agreement (Figure 2b).

### Resin selectivity – model ligands forming neutral or cationic complexes

Several experiments were designed to verify the selectivity of the ion-exchange resin using ligands that form neutral or cationic metal-ligand species that could theoretically bind to the resin, leading to an overestimate of the true free-ion concentration. Both inorganic ( $\text{Cl}^-$ ) and organic (alanine, cysteine and lysine) ligands were used to test resin selectivity.

#### Chloride

Chloride ions form several chloro-Cd complexes:  $\text{CdCl}^+$ ,  $\text{CdCl}_2^0$  and  $\text{CdCl}_3^-$ ; the cationic complex ( $\text{CdCl}^+$ ) could conceivably bind to the resin, albeit less strongly than the divalent  $\text{Cd}^{2+}$  ion. For a fixed total Cd concentration (89 nM), the concentration of NaCl was increased (3.5 to 200 mM) while simultaneously decreasing the  $\text{NaNO}_3$  concentration, thus keeping the ionic strength constant. The concentration of  $\text{CdCl}^+$  increases over this range and thus measurements of  $\text{Cd}^{2+}$  would be subject to an increasing positive interference if the resin retained some  $\text{CdCl}^+$ . Four concentrations of chloride were tested (3.5, 40, 125 and 200 mM) with three replicates for each concentration at a pH of 5.5.

Measured and calculated values of  $\text{Cd}^{2+}$  are compared in Figure 3a. Although the concentrations of the positively charged complex,  $\text{CdCl}^+$ , and neutral species,  $\text{CdCl}_2^0$ , increase with chloride concentration, these species do not affect the response of the IET; agreement between calculated and measured values for free  $\text{Cd}^{2+}$  is excellent for all chloride concentrations. Similar results were noted by Sweileh *et al.*<sup>[6]</sup>, Holm *et al.*<sup>[8]</sup> and Desnoyers<sup>[9]</sup>. However, this agreement does not necessarily imply that cationic complexes do not bind to the resin, but rather that their net contribution to the total amount of cadmium on the resin is not significant. This poor affinity of the functional groups on the resin for the mono-chloro complexes is presumably related to the reduced ionic charge and the increased effective ionic radius of  $\text{CdCl}^+$  compared to  $\text{Cd}^{2+}$ .

#### Alanine

Cadmium reacts with the amino acid alanine to form either a cationic complex,  $\text{CdAla}^+$ , or a neutral one,  $\text{Cd}(\text{Ala})_2^0$ . The formation constants for these complexes are well defined and Cd speciation can be calculated with confidence, offering a second test of the selectivity of the IET for the free-ion species. The

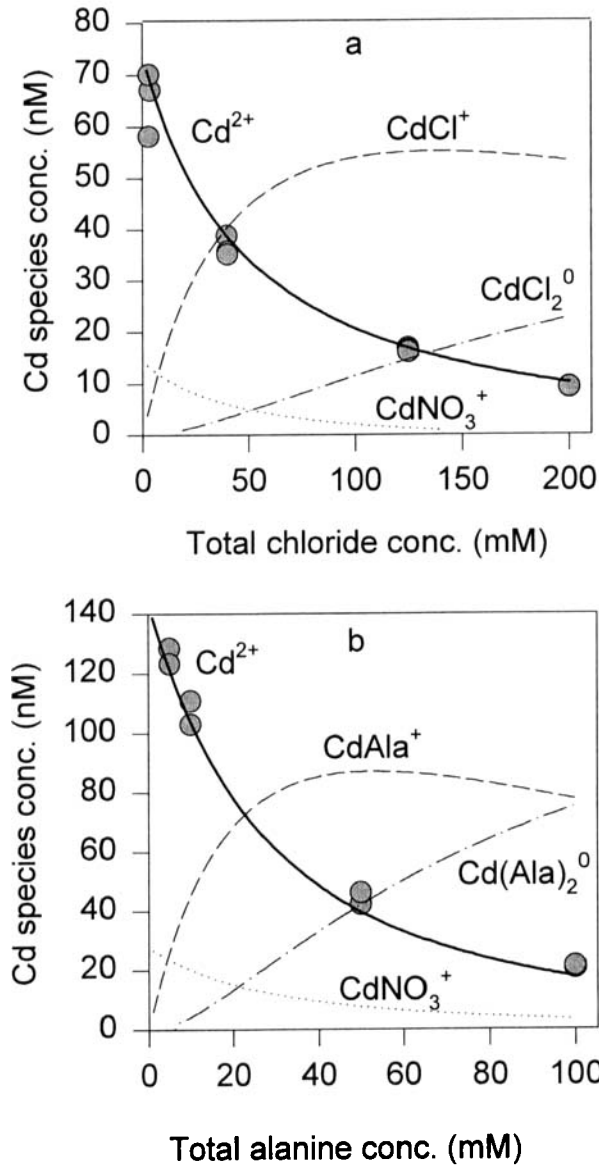


FIGURE 3 Comparison of calculated and measured concentrations of  $Cd^{2+}$  in the presence of chloride and alanine. a) Cd-Cl,  $[Cd]_{tot.} = 89$  nM, pH = 5.5; b) Cd-Alanine,  $[Cd]_{tot.} = 180$  nM, pH = 7.4; ●: Free-metal ion concentration measured with the IET; Lines represent concentrations of major species calculated with MINEQL+

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Cd concentration was kept constant at 180 nM while the alanine concentration was varied (5, 10, 50 and 100 mM) and the pH was held at 7.5. All measurements were done in duplicate. As in the case of chloride, the presence of cationic complexes did not have an impact on the response of the IET (Figure 3b). The residual charge of the CdAla<sup>+</sup> complex seems to be insufficient to lead to its retention in detectable amounts on the sulfonate resin.

### *Cysteine and lysine*

Two other amino acids, cysteine and lysine, were used to extend the search for possible interferences by cationic complexes. Both ligands contain a terminal functional group in their amino acid side chain and form either cationic or neutral complexes with Zn: ZnHCys<sup>+</sup>, ZnH<sub>2</sub>Cys<sub>2</sub><sup>0</sup>; ZnHLys<sup>2+</sup> and ZnH<sub>2</sub>Lys<sub>2</sub><sup>2+</sup>. Zinc rather than cadmium was used in these experiments because more reliable formation constants were available for Zn for these amino acids<sup>[14]</sup>. Because cysteine oxidises easily in the presence of oxygen at high pH<sup>[21]</sup>, all solutions were prepared only one hour prior to analysis. The cysteine concentrations were held constant at 50 mM (pH 4.5) or 50 μM (pH 7.0), while total zinc concentrations were varied (230 to 760 nM). In the case of lysine, its concentration was varied (10, 52 and 100 mM) while total zinc was held at 760 nM with a solution pH of 7.0. Three replicates of each solution were analysed with the IET.

As illustrated in Figure 4a, free Zn<sup>2+</sup> ion concentrations were systematically overestimated in the presence of high concentrations of lysine. Similarly, for cysteine the measured « Zn<sup>2+</sup> » concentrations were systematically higher than the calculated values at pH 4.5 (Figure 4b), whereas at pH 7 IET measured and calculated Zn<sup>2+</sup> concentrations were in more reasonable agreement (Figure 4c).

Unlike the ligands tested initially (chloride and alanine), lysine and cysteine (at low pH) seem to form metal complexes that interfere with free-ion measurements by the IET. We suspect that this difference in behavior is related to the presence of additional functionality in the lysine and cysteine side chains. For example, formation of the Zn-lysine complexes involves the carboxylic acid functional group and the α-amino group, leaving the terminal amino group free; similarly, formation of the Zn-cysteine complexes involves the carboxylate group and the β-thiol group, leaving the α-amino group free. At solution pH values close to or below their pK<sub>a</sub>s, these free amino groups will take up a proton and the metal-ligand complexes will thus carry a full positive charge on the resulting quaternary nitrogen. We suggest that these complexes can then bind to the ion-exchange resin through the protonated -NH<sub>3</sub><sup>+</sup> group, *without* disrupting the metal-ligand bonds. When the resin is subsequently extracted with an acidic solution, the metal-ligand complex will dissociate and thus contribute to an overestimation of the free-metal concentration. Even the species, ZnH<sub>2</sub>Cys<sub>2</sub><sup>0</sup>, which

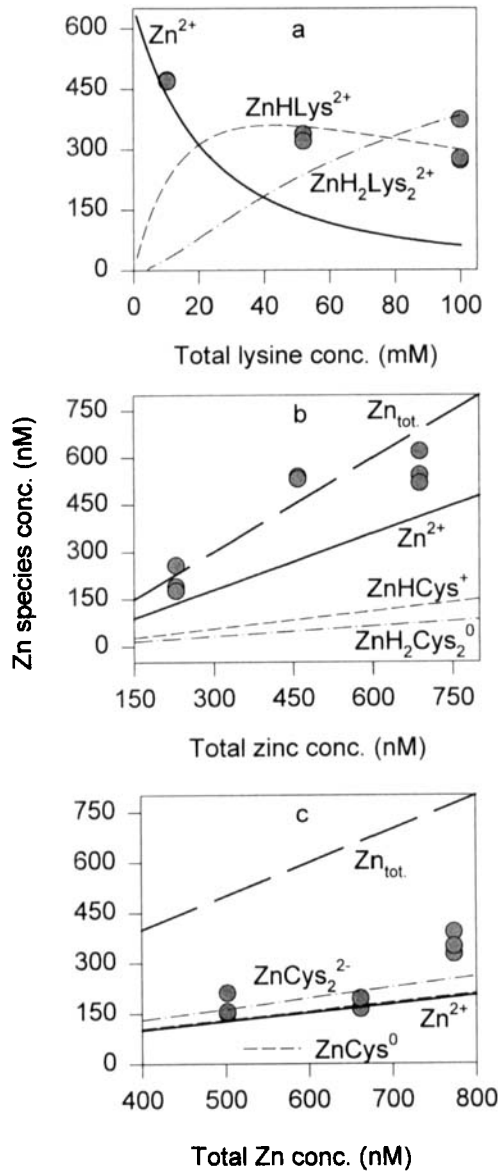


FIGURE 4 Comparison of calculated and measured concentrations of Zn<sup>2+</sup> in the presence of lysine and cysteine. a) Zn-lysine, [Zn]<sub>tot</sub> = 760 nM, pH = 7.0; b) Zn-cysteine, [Cys]<sub>tot</sub> = 50 mM, pH = 5.5; c) Zn-cysteine, [Cys]<sub>tot</sub> = 50 μM, pH = 6.9; ●: Free-metal ion concentration measured with the IET; Lines represent concentrations of major species calculated with MINEQL+



has a net null charge, possesses two protonated amine groups, so all  $ML_n$  species in the Zn-lysine and Zn-cysteine systems could have interfered. In the Zn-cysteine experiments, the overestimate of the free  $Zn^{2+}$  concentration is much less important at circumneutral pH than at acidic pH (cf. Figures 3b and 3c), as would be expected since the degree of protonation of the free  $\alpha$ -amino group also decreases as the pH is raised from 5 to 6.9.

The recent work of Lee and Hong<sup>[22]</sup> supports the suggestion that metal-lysine and metal-cysteine complexes can bind to the ion-exchange resin through a protonated- $NH_3^+$  group; they have shown that free alanine (not the metal complex) can bind to Dowex 50W-X8 resin at a pH of 6.0 through its protonated  $\alpha$ -amino group. Further support for this suggestion comes from two trials where cysteine solutions (50 mM) were run through the IET columns at pH 4.5 or 7 to test for interactions between cysteine and the resin. After equilibration with the cysteine solutions, the columns were eluted as usual with strong acid (1.5 M  $HNO_3$ ). Total sulfur concentrations in these samples, as determined by inductively coupled plasma atomic emission spectrometry (Thermo Jarrell Ash – Atomscan 25), indicated the presence of cysteine in the eluates; as expected, concentrations were higher for the columns that had been equilibrated at pH 4.5 ( $520 \pm 320 \mu M$ ) than for those run at pH 7 ( $98 \pm 30 \mu M$ ). Note, however, that Na concentrations in the eluates were indistinguishable from those observed in normal runs, indicating that the presence of cysteine had not caused any significant displacement of the Na counter-ion ( $[R-Na]$ ) from the resin; this result is encouraging, since displacement of Na could well lead to changes in the distribution coefficient  $\lambda_{o,i,pH}$  for the resin (eq. 3).

The binding of metal-amino acid complexes to cation exchange resins through protonated amine groups could also explain one of the first reported IET interferences. In their study with a similar cation exchange resin (Bio-Rad AG50W-X12), Zorkin *et al.*<sup>[23]</sup> reported «anomalous results when histidine was used as the model ligand»; their apparent  $Cu^{2+}$  concentrations were in fact higher than the total concentrations of copper used! Formation of the Cu-histidine complex involves the carboxylate group and the imidazole ring nitrogen, leaving the  $\alpha$ -amino group free; we suggest that the Cu-histidine complex binds to the cation exchange resin through the protonated  $\alpha$ -amino group, leading to an overestimate of the free  $Cu^{2+}$  concentration. The only other example of apparent interference in the presence of an amino acid is that reported by Sweileh *et al.*<sup>[6]</sup> for the Cu-glycine system; our proposed interference mechanism clearly does not apply for this simple amino acid.

### *Other cases of interference in the literature*

Sweileh *et al.*<sup>[6]</sup> also reported overestimates of  $\text{Cu}^{2+}$  concentrations in the presence of phthalate and salicylate as ligands. These ligands both contain an aromatic ring and form neutral complexes with Cu. The recent results obtained by Lee and Hong<sup>[22]</sup>, demonstrating non-ionic interactions of phenylalanine and tyrosine with Dowex 50W-X8, suggest that these interferences were likely due to the adsorption of the neutral metal complexes to the hydrophobic domains of the resin.

### **DOM-resin interactions**

The Dowex cation exchange resin shares the same backbone (styrene divinylbenzene copolymer) as the XAD resins that are used to extract organic matter from natural waters<sup>[19,24,25]</sup>. Two experiments were designed to verify if humic or fulvic acid adsorbs to the Dowex resin, and to determine if this adsorption has any effect on the distribution coefficients for Cd or Zn in the IET. Although both natural dissolved organic matter and the Dowex resin are negatively charged, and thus repel each other, fulvic and humic acids also possess hydrophobic domains<sup>[19]</sup> and this might conceivably lead to a non-ionic interaction, as pointed out by Lee and Hong<sup>[22]</sup>.

To check for adsorption of DOM onto the resin, batch adsorption experiments were carried out at pH 4.5. A decrease in light absorption for the solutions exposed to the resin would have demonstrated adsorption of the DOM by the resin. However, the light absorbance of all solutions containing the resin increased slightly. When  $20 \text{ g}\cdot\text{L}^{-1}$  of resin were used, the absorbance increased by 6% (0.003 units) for fulvic acid and 7% (0.006 units) for humic acid; this increase corresponded to the increase in absorbance detected in the DOM-free control experiment (0.004 units). The increase in the control flasks was not unexpected<sup>[9]</sup> and presumably results from leaching of light-absorbing organic material from the resin. After correcting for the leaching of the resin, there was no significant differences in absorption between solutions with and without resin for either fulvic or humic acid. Since the test conditions were chosen to favour adsorption (low pH; enormous quantity of resin used in this test, up to  $20 \text{ g}\cdot\text{L}^{-1}$ , compared to the amount used for the IET (300 mg for the manual method and 7.6–7.8 mg for the miniaturised method); long exposure time (8 h compared to a few minutes for the IET)) we can conclude that no significant decrease in DOM concentration should occur during analyses of aqueous samples containing natural organic matter. Note in passing that if the material leached from the resin can complex metals, then the use of batch ion-exchange experiments<sup>[7–9]</sup> to deter-

mine free-metal ion concentrations becomes questionable. This caveat does not apply to measurements made in columns since resin leachates would not accumulate but rather be evacuated continuously.

Although we could detect no decrease in DOM concentration on contact with the resin, only a very small amount of adsorbed material might conceivably alter the resin's ion-exchange behavior (distribution coefficient). Cantwell *et al.*<sup>[4]</sup> showed that there was no trend in the Ni distribution coefficient when increasing amounts of DOM were introduced onto the column prior to a standard solution. Similarly, we designed an experiment where four columns were treated with a 500 mL solution of fulvic acid ( $10 \text{ mg}\cdot\text{L}^{-1}$ ) and sodium nitrate (0.20 M) at pH 5.5 prior to analyzing standard solutions containing 38 and 22 nM of Zn and Cd respectively. Another control group of four columns was treated with the same amount of solution but without any DOM added, followed by the same standard solutions. From the metal concentrations in the eluates, the distribution coefficients were calculated and compared between the two sets of columns. There were no significant differences ( $P < 0.05$ ) between the two groups. The average distribution coefficients ( $\pm$  standard deviation,  $n=4$ ) for treated and untreated columns were  $0.52 \pm 0.01$  and  $0.52 \pm 0.02 \text{ L}\cdot\text{g}^{-1}$  for Cd and  $0.45 \pm 0.01$  and  $0.46 \pm 0.02 \text{ L}\cdot\text{g}^{-1}$  for Zn respectively. Clearly treatment of the resin with fulvic acid does not modify its exchange properties. This result suggests that free-metal ion concentrations can be determined without adsorption artifacts in complex aqueous media containing natural DOM.

### Speciation of Cd and Zn in solutions containing fulvic acid

Having shown that the IET could be used in the presence of natural DOM, we determined free  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  concentrations in solutions containing  $10 \text{ mg}\cdot\text{L}^{-1}$  of Laurentian fulvic acid. These measurements were used to design laboratory bioassays conducted with the same fulvic acid as a natural metal ligand. This concentration of fulvic acid is representative of concentrations observed during spring snowmelt in rivers of the Côte-Nord region of Quebec, Canada<sup>[26]</sup>. The range of total metal concentrations corresponded to those used for the short-term toxicity tests: 4.4, 22, 44, 57, 89 and 320 nM Cd and 76, 150, 310 and 610 nM Zn. The measured speciation results at pH 5.5 were compared to predictions of the Windermere Humic Acid Model (WHAM) developed recently by Tipping<sup>[18]</sup>. Solutions were prepared in duplicate (with two exceptions: for total Cd concentrations of 57 and 320 nM, the numbers of replicates were 6 and 12 respectively), and left to equilibrate overnight before measurements were taken with the manual IET.

Measured free  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  concentrations are plotted in Figure 5, along with the predicted values as calculated with WHAM – agreement between predicted and measured values is clearly erratic. At pH 5.5 the calculated  $\text{Zn}^{2+}$  concentrations fall well below the measured values (Figure 5a), whereas calculated free  $\text{Cd}^{2+}$  is quite close to the measured concentrations (Figure 5b); at pH 7 this agreement for  $\text{Cd}^{2+}$  breaks down, however, and the calculated values greatly exceed the measured values (Figure 5c). We speculate that the model may not account for the «unwinding» of the fulvic acid molecule that occurs when sites ionize at higher pH, repelling one another; this change in the conformation may well affect the affinities of the metal-complexing sites. The divergence between measured and predicted  $\text{Cd}^{2+}$  values at pH 7 suggests that the apparent agreement at pH 5.5 was fortuitous, and argues against the incautious application of WHAM to uncalibrated metal-DOM systems.

It is also instructive to consider the relative affinities of fulvic acid for Cd and Zn: for solutions at pH 5.5, WHAM predicts that fulvic acid should complex Zn to a greater extent than Cd, whereas our measured values at pH 5.5 indicate the opposite trend (compare solid lines in Figures 5a and 5b). The WHAM predictions are intuitively reasonable, given the relative “hardness” of the zinc cation and the predominance of Ocontaining functional groups in fulvic acids. The conflicting empirical results obtained here suggest that at low Cd concentrations other functional groups within the fulvic acid, such as thiols, may be participating in the complexation of Cd; sulfur has been reported to represent 0.5–1.8% of the elemental composition of natural DOM<sup>[27,28]</sup>. If these high affinity sites are to control the speciation of Cd, the fulvic acid must be in considerable excess with respect to Cd. To obtain an indication of the relative concentrations of Cd and fulvic acid, we used the degree of site occupation proposed by Buffle *et al.*<sup>[29]</sup>. When the ratio of complexed metal to total site concentrations is less than 0.1, «complexant properties are dominated by those of minor sites». To determine this ratio, the highest concentration of complexed Cd (~ 200 nM) was used. Using a conservative estimate of charge density of Laurentian fulvic acid<sup>[30]</sup> of  $5.8 \text{ meq}\cdot\text{g}^{-1}$  and multiplying by the mass concentration used ( $10 \text{ mg}\cdot\text{L}^{-1}$ ), we get a concentration of  $0.058 \text{ meq}\cdot\text{L}^{-1}$ . If we further assume a one-to-one relationship between Cd and the fulvic acid, the degree of site occupation can be estimated as 0.0034, which is about 30 times lower than the threshold of 0.1. Thus, at the concentrations of Cd used in our experiments, conservative calculations indicate that the fulvic acid was in excess and that minor sites, such as thiol groups, would have dominated the speciation.

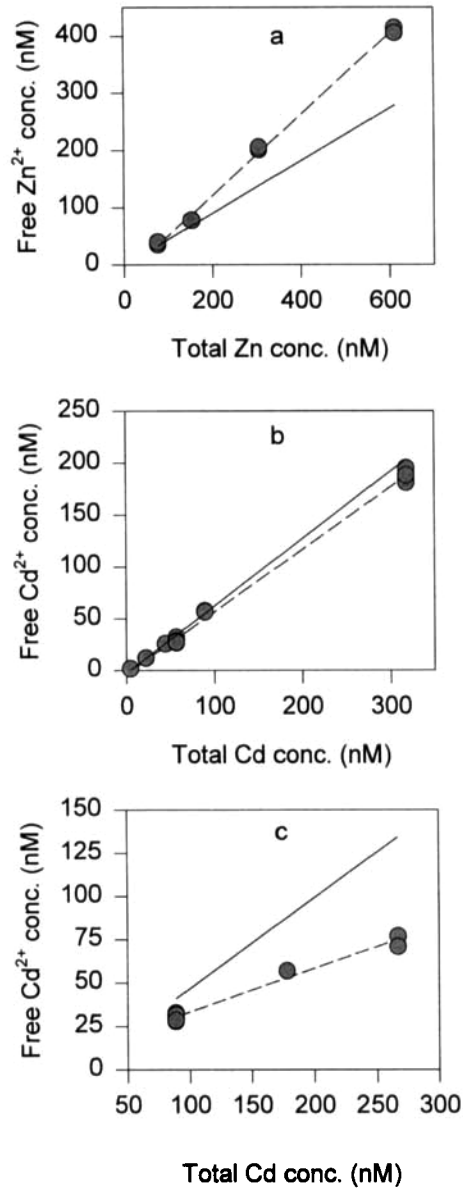


FIGURE 5 Comparison of calculated and measured concentrations of  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  in the presence of  $10 \text{ mg}\cdot\text{L}^{-1}$  Laurentian fulvic acid a) Zn-FA, pH = 5.5; b) Cd-FA, pH = 5.5; c) Cd-FA, pH = 7.0; ●: Free-metal ion concentration measured with the IET (dotted lines represent linear regressions); Full lines represent concentrations of free-metal ion predicted using the WHAM model

## CONCLUSION

In the fields of aquatic geochemistry and toxicology, there is a critical need for reliable analytical methods to measure free-metal ion concentrations in complex aquatic matrices, especially in those containing natural dissolved organic matter (fulvic and humic acids). Equilibrium cation exchange offers a simple but very promising approach to this analytical problem. Advantages of the technique include its simplicity and sensitivity, its inherent capacity for simultaneous multi-element determinations, and its apparent insensitivity to DOM adsorption artifacts. Adsorption of natural DOM on the cation exchange resin is not quantitatively significant at pH values equal to or greater than 4.5 nor does it affect the distribution coefficients for  $\text{Cd}^{2+}$  or  $\text{Zn}^{2+}$ . The selectivity of the technique for divalent free-metal ions is good, in that the residual positive charge on an initially divalent metal after complexation (e.g.,  $\text{CdCl}^+$ ) is insufficient to cause a significant interference in the eluant signal. Positive interferences do occur, however, in the presence of complex amino-acid ligands, where the metal-ligand complex contains a positively charged quaternary ammonium group (e.g., M-lysine and M-cysteine systems). Possible uses of the IET would be to monitor free-metal ion levels in bioassay experiments using natural DOM, to determine formation constants and complexation capacities of complex organic ligands (e.g., polypeptides) or to determine free-metal ion concentrations in contaminated surface waters.

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### References

- [1] A. Tessier, J. Buffle and P.G.C. Campbell. In: *Chemical and Biological Regulation of Aquatic Systems* (J. Buffle and R.R. DeVitre, eds.; Lewis, Boca Raton FL, 1994) pp. 197–230.
- [2] F.M.M. Morel. *Principles of Aquatic Chemistry* (Wiley, New York, 1983) p. 301.
- [3] P.G.C. Campbell. In: *Metal Speciation and Bioavailability in Aquatic Systems* (A. Tessier and D.R. Turner, eds.; Wiley, New York, 1995) pp. 45–102.
- [4] F.F. Cantwell, J.S. Nielsen and S.E. Hrudrey, *Anal. Chem.*, **54**, 1498–1503 (1982).
- [5] J. Treit, J.S. Nielsen, B. Kratochvil and F.F. Cantwell, *Anal. Chem.*, **55**, 1650–1653 (1983).
- [6] J.A. Sweileh, D. Lucyk, B. Kratochvil and F.F. Cantwell, *Anal. Chem.*, **59**, 586–592 (1987).
- [7] J. Werner, *Sci. Tot. Environ.*, **62**, 281–290 (1987).
- [8] P.E. Holm, T.H. Christensen, J.C. Tjell and S.P. McGrath, *J. Environ. Quality*, **24**, 183–190 (1995).
- [9] C. Desnoyers. (M.Sc. Thesis, INRS-Eau, Université du Québec, T-185, 1995), 133 p.
- [10] H. Ren and B. Kratochvil, *Intern. J. Environ. Anal. Chem.*, **60**, 303–312 (1995).
- [11] H. Ren and B. Kratochvil, *J. Chem. Eng. Data*, **40**, 1091–1093 (1995).

- [12] N.M. Price, G.I. Harrison, J.G. Hering, R.J. Hudson, P.M. Nirel, B.Palenik and F.M.M. Morel, *Biological Oceanography*, **6**, 443–461 (1989).
- [13] W.D. Schecher and D. McAvoy, Environmental Research Software, Version 3.01, Hallowell, ME (1994).
- [14] R.M. Smith, A.E. Martell and R.J. Motekaitis, NIST Standard Reference Database 46, Version 3.0, U.S. Department of Commerce, Gaithersburg, MD (1997).
- [15] EPA (Anonymous), *Environ. Sci. Technol.*, **29**, 66a (1995).
- [16] C. Fortin and J. Gauthier, INRS-Eau, Université du Québec, Report No. RI-136 (1995), 13 p.
- [17] S.M. Serkiz, J.D. Allison, E.M. Perdue, H.E. Allen and D.S. Brown, *Water Res.*, **30**, 1930–1933 (1996).
- [18] E. Tipping, *Computers & Geosciences*, **20**, 973–1023 (1994).
- [19] E.M. Thurman. *Organic Geochemistry of Natural Waters* (W. Junk Publishers, Boston, 1985), 497 p.
- [20] M. Schnitzer. In: *Soil Organic Matter* (M. Schnitzer and S.U. Khan, eds.; Elsevier, Amsterdam, 1978), pp. 1–64.
- [21] A. Fontana and C. Toniolo. In: *The Chemistry of the Thiol Group* (S. Patai, ed.; Wiley, New York, 1974), pp. 271–321.
- [22] K. Lee and J. Hong, *Reactive & Functional Polymers*, **28**, 75–80 (1995).
- [23] N.G. Zorkin, E.V. Grill and A.G. Lewis, *Anal. Chim. Acta*, **183**, 163–177 (1986).
- [24] G.R. Aiken. In: *Humic Substances and Their Role in the Environment* (F.H. Frimmel and R.F. Christman, eds.; Dahlem Workshop Reports, Wiley, New York, 1988), pp.15–28.
- [25] G.E. Batley. In: *Trace Element Speciation. Analytical Methods and Problems* (G.E. Batley, ed.; CRC Press, Boca Raton, FL, 1989), 350 p.
- [26] P.G.C. Campbell, H.J. Hansen, B. Dubreuil and W.O. Nelson, *Can. J. Fish. Aquat. Sci.*, **49**, 1938–1952 (1992).
- [27] M.J. Morra, S.E. Fendorf and P.D. Brown, *Geochim. Cosmochim. Acta*, **61**, 683–688 (1997).
- [28] M. Schnitzer and H. Kerndorff, *Water, Air and Soil Pollution*, **15**, 97–108 (1981).
- [29] J. Buffle, R.S. Altmann, M. Filella and A. Tessier, *Geochim. Cosmochim. Acta*, **54**, 1535–1553 (1990).
- [30] M.A. Glaus, W. Hummel and L.R. Vanloon, *Anal. Chim. Acta*, **303**, 321–331 (1995).