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Determination of dynamic metal complexes and their diffusion coefficients in the presence of different humic substances by combining two analytical techniques

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Abstract

The combined use of a competing ligand exchange (CLE) method and a diffusive gradients in thin films (DGT) technique in a quasi labile system provides a better understanding of dynamic metal (Cu and Ni) complexes in the presence of humic substances of different origins. The CLE and DGT techniques provide total labile (dynamic) metal complexes (Cu and Ni complexes) and their dissociation rate constants in environmental systems. In this study, DGT technique was found to estimate lower concentrations of labile metal complexes than CLE technique in the same systems. This discrepancy in result was because of the diffusion controlled metal flux (towards the binding resin gel) in the diffusive gel of DGT. The interactions of Cu and Ni with humic acids are stronger than the interactions of them with fulvic acid and natural organic matter. Changes in lability of Ni and Cu complexes (complexed with humic substances of different origins) with the changing analytical detection window indicate that labile complexes of these metals were formed with different binding sites with diverse binding energies in the humic substances. The combination of these two techniques was found to be very useful in determining diffusion coefficients of labile metal- humate complexes in quasi-labile systems. The values of diffusion coefficients of labile Ni and Cu complexes determined in this study are in good agreement with a limited set of results from the literature. This finding is novel and can be very useful in further improving our understanding of the metal–humate interactions in natural environments.

Key words: Ni speciation; Cu speciation; DGT and CLE; Diffusion coefficient; Humic substances

Introduction

Development of dynamic metal speciation analysis is essential for reliable predictions of bioavailability and risk assessment of metal pollution in aquatic environments (Campbell 2006; McGeer 2003). Humic substances modify the speciation of metals in natural system and influence the capacity of natural waters to sustain life after metal pollution events. The presence of continuous distribution of metal binding sites with different binding energies in humic substances poses considerable challenge in determining reliable physicochemical parameters which can describe metal speciation in aquatic systems (Ruzic, 1996; Chakraborty 2006a; ,2006b, 2007, 2010;).

One of the most important metal speciation parameters, which helps elucidating the mass transport, complexation kinetics, bioavailability and toxicity of a metal species in an environmental system, is the diffusion coefficient (Li 2005; Chakraborty 2010). However, determination of the diffusion coefficient of metal species is extremely difficult in natural (quasi-labile) systems due to the presence of heterogeneous humic substances (Oelkers 1991; Furukawa 2008;).

Direct determination of diffusion coefficient of metal complexes in quasi labile systems is scarce. Determination of average diffusion coefficient of dynamic metal complexes has been reported in the literature by using different techniques such as stripping voltammetry (SV) (Domingos 2004), gel permeation chromatography (Chin 1994; Perminova 2003), ultrafiltration (Hoffmann 2000; Forsberg 2006;), diffusion through activated carbon columns (Cornel 1986), and dynamic light scattering (DLS) (Chakraborty 2010). It has been well documented that the use of a single technique may not be sufficient to obtain complete information about the metal speciation dynamics in complex aquatic systems. A combination of two or more appropriate techniques, depending on their time scale of measurement and analytical window, will provide more useful information on metal speciation (Ure 1995; Chakraborty 2009, 2010, 2011, 2012, 2013; Michalke 2010). In the present study, The diffusive gradient in thin films (DGT) technique was used in combination with the competing ligand exchange (CLE) method to provide better information on speciation of dynamic metal complexes of Cu and Ni and their diffusion coefficients in presence of humic substances from different origins.

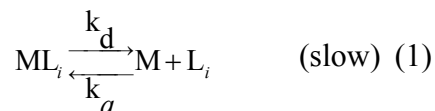
The CLE method, a bulk equilibrium technique involving competition between natural ligand and added ligand (L_{ad}) for metals in aquatic system, measures dynamic metal complexes (effectively weaker metal complexes than the $M-L_{ad}$ complexes) and their dissociation rate constants (k_d) in aquatic system. The DGT technique has also been reported to determine labile metal complexes in aquatic system. In DGT,

a suite of metal complexes (including free metal ions, and other metal complexes that dissociate within the diffusive layer) accumulate in the binding resin gel. The lability and diffusion coefficients of the penetrating complexes determine the amount of metal accumulation in the binding resin in the DGT. The combined use of both techniques is expected to provide useful information of dynamic metal speciation in aquatic environment. Identical results cannot be expected from both techniques because they are based on different principles. However, in the same detection window (sensing the similar complexes), comparison of the data (obtained by these two techniques) can provide a better understanding on the dynamic metal speciation. The differences in the concentrations of labile metal complexes determined by these two techniques are due to the change in diffusion coefficients of metal complexes in the diffusive layer in DGT. In this study, the DGT technique was used in combination with the CLE method to determine speciation of dynamic Cu and Ni complexes and their diffusion coefficients in presence of humic substances (of different origin) in quasi labile systems.

Theory

Competing ligand exchange (CLE) method

The kinetic model developed by Olson and Shuman (Olson 1985) has been adapted (Chakraborty 2006a, 2006b, 2012a, 2012b) to study the dissociation of a metal complex, ML , where M is a metal ion and L is a macromolecular, polyfunctional complexant, such as a humic substance, having binding sites of different chemical affinities (the charges on M and L have been omitted for simplicity). In the complexant, L , the metal M is bound to multiple sites in L , all of which dissociate independently and simultaneously at a rate that depends on the nature of the functional group, its position on the macromolecules, and the residual charge. The dissociation is assumed to be first-order. Consider an aqueous mixture of n components, in which each component, designated ML_i , exists in equilibrium with its dissociation products:



Where, where k_a and k_d are the rate constants for complex association and dissociation respectively and the thermodynamic stability constant, K , equals ka/kd .

The CLE method uses chemical competition of natural ligand with competing ligand (in this study, Chelex 100 chelating resin (solid) as the competing ligand) for M. When Chelex-100 is added in sufficient excess, it reacts with M as follows:



The model assumes that

1. Reaction (2) is much faster than reaction (1), and
2. $[\text{Chelex}] \gg [\text{M}]$

Because $[\text{Chelex}] \gg [\text{M}]$, reaction (2) is pseudo-first-order. Since k_f is large, as has been determined by graphite furnace atomic absorption spectroscopy (GFAAS), and with Chelex-100 in sufficient excess, the condition $k_f [\text{Chelex}] \gg k_a [\text{L}]$ holds, and the overall reaction



is shifted to the right. Hence, the rate of formation of the M-Chelex complex is determined by the rate of dissociation of ML in reaction (1), and the rate expression is simply:

$$\frac{dC_{\text{M-Chelex}}}{dt} = -\frac{dC_{\text{ML}_i}}{dt} = k_d C_{\text{ML}_i} \quad (4)$$

If each complex, ML_i , undergoes, independently and simultaneously, a first-order or pseudo-first-order dissociation reaction, the sum of the concentrations of all components of the complex ML remaining undissociated at time, t , $C(t)$ can be described as:

$$C(t) = \sum_{i=1}^n C_i^{\circ} \exp(-k_i t) \quad (5)$$

where C_i° is the initial concentration of ML_i , the i^{th} component. The $C(t)$ is measured using GFAAS by measuring the uptake of metal ion by Chelex 100 as a function of time.

The profiles of dissolved metal concentrations under different conditions a) fully labile; b) inert; and c) quasi-labile are shown in Figure 1.

The *lability criteria* in CLE method is given below.

The concept of *lability* describes the ability of complexes to maintain equilibrium with the free metal ion, M, within the context of an ongoing interfacial process in which a particular species, usually M, is consumed. Consider the simplest case of a metal complexation reaction as described in Eq 1.

Under conditions of sufficient excess ligand (L) over metal (M), the association reaction is quasi-monomolecular with rate constant $k'a = k_a c_L$. On time scales, t , much larger than the characteristic lifetimes of M ($1/k'a$) and ML ($1/k_d$), a given metal ion undergoes frequent interchange between M and ML. The complex system is then sufficiently dynamic to maintain bulk equilibrium and obeys the double condition (Gaabass et al., 2009)

$$K'a t, k_d t \gg 1 \quad (6)$$

In the usual situation of practical interest, $K' (= K c_L) k'a/k_d > 1$, so eq 6 reduces to $k_d t \gg 1$.

Thus, metal complexes will be called labile if their $k_d t \gg 1$.

Diffusive Gradients in Thin Films Technique (DGT)

DGT is composed of three layers: (1) a 0.45- μm membrane filter which separates the dissolved phase from the particulate phase; (2) a diffusive gel layer comprised of hydrous acrylamide-polymer chains linked with bis or agarose cross-linkers, which controls the transport of metals (i.e., flux) from the bulk solution; and (3) a metal-binding layer composed of polyacrylamide gel embedded with Chelex 100, which serves as a sink for metals that diffuse through the polyacrylamide gel layer.

The geometry and construction of a DGT device has been described by Zhang and Davison (Zhang 1995). DGT is based on the coupled diffusion of the metal complex, ML, and the free metal ion, M, from the sample medium through a stagnant boundary layer, where only M is bound by the receiving phase. This shifts the equilibrium between M and ML, inducing a steady-state concentration gradient within the boundary layer as shown in Figure 1. ML is labile when the formation and dissociation kinetics are sufficiently fast that the total metal flux towards the binding phase is controlled exclusively by the coupled diffusion of M and ML. Labile metals in the bulk solution diffuse through the stagnant boundary layer and are concentrated in the resin. Metal complexes not able to dissociate within the boundary layer are non-labile. Intermediate between these two limiting conditions is the general case (quasi-labile) in which ML is only able to dissociate partially before reaching the binding phase. Puy et al have suggested that a particular dynamic feature of the DGT devices stems from the fact that the reaction layer is not only present in the gel domain but also extends into the resin domain.

The accumulated mass (m) of M in the resin gel can be expressed by the following equation:

$$m = \frac{(C_M D_M + C_{ML} D_{ML})}{d_g} A t$$

where, D_M , D_{ML} , C_M and C_{ML}^* , d_g , A and t represent the average diffusion coefficients of metal ion and metal complexes, the bulk concentration of free metal ion and dynamic metal species, diffusive gel thickness, area of the exposed window in DGT device, and the deployment time.

Following deployment of the DGT device in solution, free metal ions and metal complexes diffuse through the diffusive gel. Metal complexes that can dissociate within the time scale of measurement, bind to the binding resin (Gaabass 2009; Gimpel 2003; Zhang 1999, 1995). Steady-state concentration profiles for the coupled diffusion of M and ML; a) fully-labile, b) inert and c) quasi-labile system in DGT are depicted in Figure 1.

The flux of metal under steady state for any lability can be represented by the following equation 6 (Puy 2012):

$$J_{flux} = \frac{D_M c_M^*}{d_g} + \frac{D_{ML} c_{ML}^*}{d_g} \zeta \quad (7)$$

where, D_M , D_{ML} , C^* , d_g and ζ represent the average diffusion coefficient of the metal ion and metal complexes, the bulk concentration of dynamic metal species, diffusion layer thickness in the solution, the diffusive layer thickness, and the degree of lability respectively.

The configuration of the DGT device used in this study had the higher diffusive gel thickness than that of the resin layer (r). Under this condition, a complex is considered as labile if $r > (D_{ML}/k_d)^{1/2}$, where D_{ML} is the diffusion coefficient of the metal complex and k_d its dissociation rate constant (Puy 2012).

Comparison of DGT and CLE method

The combined use of CLE and DGT in a quasi labile system is expected to provide better understanding of dynamic metal complexes in the system. The CLE technique is capable of providing total labile (dynamic) metal complexes in a quasi-labile system and DGT technique is expected to sense the same concentrations of labile metal complexes in the same system as the kinetics of dissociation of labile metal complexes (obtained from the CLE method) is expected to be fast. However, the metal flux (towards the binding resin) is limited by diffusion of the complex in diffusive gel (DGT). Therefore, results will not be comparable. However, the comparison of data from the two techniques can be very useful in determining diffusion coefficients of labile metal complexes in a quasi labile system.

In DGT, the use of a diffusive gel layer in front of the binding resin layer provides a well-defined diffusive layer which constrains mass transport (Chakraborty 2009; Gaabass 2009), so that a precise

effective measurement time can be calculated and used to define the kinetic detection window. It has been reported that the typical time (τ) necessary for attaining a steady-state profile in DGT with a gel layer of 400–1000 μm thicknesses for metal complexes with an overall diffusion coefficient of $10^{-10}\text{m}^2\text{s}^{-1}$ are in the order of d_g^2/D , i.e. 10^3 – 10^4 s. The CLE/GFAAS technique, a bulk equilibrium technique (Gaabass et al., 2009), needs time to attain the chemical equilibrium in the bulk solution depending on the characteristics of the complexing ligand. If the timescale required for attaining the steady-state in the DGT technique is similar to the time required to attain chemical equilibrium in CLE for the same metal in the same sample, then the results of the two techniques can be compared as follows.

$$\frac{DGT_{labile}}{CLE_{dynamic}} = \frac{D_M C_M + D_{ML} C_{ML}}{D_M C_{T,dynamic}} \quad (8)$$

where, D_{ML} and D_M represent the diffusion coefficient of dynamic metal complexes and free metal respectively, C_{ML} and C_M represent the concentration of dynamic metal complexes and free metal ion respectively, and $C_{T,dynamic}$ represents the total dynamic metal complexes determined by the CLE/GFAAS technique.

Materials and methods

Humic substances: Four different types of humic substances were used in this study. These include soil humic acid from Sigma Aldrich now denoted: SAHA. The well characterized Nordic aquatic humic acid (Catalogue No. 1R105H) (NAHA), Nordic aquatic fulvic acid (Catalogue No. 1R105F) (NAFA) and Suwanee River natural organic matter (Catalogue No. 1R101N) (SRNOM) were also used. These latter three were obtained from International Humic Substance Society (IHSS; MN, USA). The stock solutions of $\sim 1.00\text{ g}\cdot\text{dm}^{-3}$ humic substances were prepared by dissolving approximately 0.1 g of the humic substances in 100 cm^3 of ultrapure water and NaOH at pH 10. The pH was then adjusted to 8.0 with ultrapure HNO_3 . The resulting humic substances solution was stored in the dark at 4°C .

Humic acid solutions composition

The elemental compositions, concentrations of carboxylic and phenolic–OH and carbohydrate in SAHA, NAHA, NAFA and SRNOM, are presented in Table 1. The atomic ratios of 1.04 (H/C), 0.42 (O/C), and 0.012 (N/C) in SAHA have been reported and these atomic ratios are within the values reported typically for soil HAs, with the exception of the N/C ratio (Rodrigues 2009). In addition, the molar ratio

of protons on saturated and unsaturated carbon atoms is approximately 2.1 : 1 in the SAHA (Rodrigues 2009) compared to other humic substances used in this study. SAHA is a soils humic acid and is quite distinct from the other three aquatic humic substances used in this study. In general, it is important to note here that the present experiments were performed with humic substances from different origins and composition.

Synthetic solutions

Four sample solutions were prepared (separately) in ultrapure water containing $5.0 \text{ mg}\cdot\text{dm}^{-3}$ of four different types of humic substances (SAHA, NAHA, NAFA, SRNOM) and $5\times 10^{-7} \text{ mol}\cdot\text{dm}^{-3}$ of Ni(II) and another four solutions were made with $5\times 10^{-7} \text{ mol}\cdot\text{dm}^{-3}$ of Cu(II). The metal concentrations used here fall within environmentally relevant ranges. The pH of the solutions was adjusted to 7.0 ± 0.1 using ultrapure NaOH and HNO₃, and the ionic strength was maintained at $10 \text{ mM}\cdot\text{dm}^{-3}$ with KNO₃. The solutions were left to equilibrate for 72 h in the dark prior to analysis. The pH of the solutions was checked again before analysis and found to be the same.

Kinetic extraction procedure

Kinetic speciation studies of Cu and Ni (II)-humate complexes were carried out using Chelex 100 as the competing ligand and graphite furnace atomic absorption spectroscopy (GFAAS) to monitor the change of dissolved metal concentration in the solutions.

Two grams of Chelex 100 was added to 200 cm^3 of sample solution (1% w/v) in a 500-cm^3 , cylindrical Teflon reactor. The sample solution was stirred continuously with a Teflon-coated stir bar. Two- cm^3 aliquots were collected over a 3-h period using a disposable plastic syringe equipped with a $0.45\text{-}\mu\text{m}$ syringe filter to separate the Chelex 100 resin from the sample solution. All containers were made with Teflon, and were cleaned with 10% (v/v) nitric acid and rinsed with ultrapure water at least three times before use. The dissociation rate constants were obtained by fitting the kinetic data to Eq. 3 by nonlinear regression analysis using SigMPlot 10 (Systat Software).

DGT measurements

Polyacrylamide (PAM) gel, binding resin gel (using Chelex-100), and DGT holders were purchased from DGT Research Ltd. (UK). The DGT samplers were assembled following the previously described procedures (Zhang and Davison, 1995), where using $0.45 \text{ }\mu\text{m}$ pore-size cellulose acetate filter (Whatman, USA) was used as a protective membrane (0.013 cm thickness). The thicknesses of the PAM gels were

0.04, 0.08 and 0.16 cm respectively. DGT devices were deployed in the synthetic solutions having sufficient volume to ensure that the depletion of metal concentration by the DGT devices was negligible. After retrieving the DGT devices from their deployments, Chelex binding gel layers were removed and placed in 1.5 cm³ plastic vials. One cm³ of ultrapure nitric acid was then added to the vials, and the test solutions were left for 24 hrs to leach out all bound metals from the Chelex resin layer into the nitric acid. A graphite furnace atomic absorption spectrometer (Perkin–Elmer *AAnalyst* 800) was used to determine the concentration of accumulated metals in the Chelex resin, from which the labile metal fraction was calculated.

Instrumentation

A PerkinElmer Model *AAnalyst* 800 atomic absorption spectrometer with an AS-800 autosampler was used. All the measurements were based on integrated absorbance and were performed using a Zeeman-effect background correction system. The determination of Cu and Ni was performed at 324.8 nm and 232.0 nm respectively using a PerkinElmer hollow cathode lamp. THGA tubes (PerkinElmer) with an integrated L'vov-type platform were used in the Cu and Ni determination. Argon (PRAXAIR, Canada) was used as a protective gas throughout. A volume of 20 µL of sample solution was injected into the furnace for metal analysis.

QA/QC

Quality control for metal analyses included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of the Certified Reference Standard, NIST 1640. A prior set of samples was re-run if the analysed value differed from the Reference Standard's certified value by > 10%. Blank measurements were performed for all the humic substances. The concentrations of these two metals in the blank solutions were below the detection limit of the GFAAS.

Non-linear regression analysis for kinetic measurement

Analysis of experimental data to obtain physical parameters is a crucial point in the kinetic speciation method. In this paper, data was fitted to the kinetic model by non-linear regression analysis using the Marquadt–Levenberg algorithm. The minimum number of parameters required to accurately fit the data was determined by finding the number of components in which the sum of squares of the weighted residuals shown below achieved a minimum value.

$$\text{Sum of squares of the weighted residuals} = \sum \left[\frac{C(t) - C_T(t)}{C(t)^{1/2}} \right]^2$$

where $C(t)$ is the experimental value and $C_T(t)$ is the calculated value using the parameters obtained from the regression analysis. For a polyfunctional complexant such as humic substances, the number of components is not a simple issue. The small number of components with their specific rate constants may not accurately describe the chemistry of the binding sites of the humic substances. A binding site may have a range of binding energy because of the heterogeneous nature of humic substances. This in turn will lead to a distribution of values for the dissociation rate constants for the complexes. Nevertheless, the specific rate constants may represent an average value for a group of complexes on a particular site.

Results and Discussion

Kinetic speciation of Cu and Ni by CLE

Kinetic speciation data for Ni and Cu in presence of different humic substances (individually) in aquatic environment (obtained by CLEM/GFAAS) are presented in Figure 2. The relative concentrations (in terms of percentage with respect to the initial concentration) of dissolved Ni and Cu (in solution) are plotted as a function of time.

The data in Figures 2 (a) and (b) were fitted to the kinetic model by nonlinear regression analysis, as described elsewhere (Chakraborty 2013; 2011; Gaabass 2009; Hassan 2006; Mandal 1999; Sekaly 1999a; Sekaly 1999b), to obtain the relative concentration of each kinetically distinguishable components and their associated dissociation rate constants. Two kinetically distinguishable components were observed in each of the trend lines presented in Figures 2 (a) and (b). The steep slope at the beginning of each trend line in Figures.2 (a) and (b) can be attributed to rapidly dissociating inorganic/organic complexes (c_1) of Ni and Cu (with $k_{d1} \sim 10^{-3} \text{ s}^{-1}$) and followed with the uptake of Ni^{2+} , Cu^{2+} free metal ions by chelex-100. The later part of the trend lines, which run parallel to x-axis, can be attributed to the stronger metal-humics complexes (c_2) with slow dissociation rate constants (with $k_{d2} \sim 10^{-5}$ to 10^{-6} s^{-1}).

The experimental data from the kinetic extraction study were fit to a two components model because it was the simplest model (i.e. minimum number of components) that gave an adequate statistical and visual fit to the data (i.e. good representation of the system). Although use of more components in the

model often statistically fit the data better, the additional components were often of negligible concentration or had a high degree of uncertainty. The percentage of dynamic and inert complexes of Ni and Cu and their corresponding dissociation rate coefficients are presented in Table 2.

The Pearson correlation matrix between the dynamic and inert complexes of Ni and Cu with the –S, -N, -COOH, phenolic-OH concentrations of the humic substances is presented in Table 3. A strong correlation was found between the dynamic complexes of Ni and Cu with the average concentrations of –COOH and phenolic-OH groups. A strong positive correlation was found between inert complexes and –S and –N content of the humic substances.

It has been estimated that in humic substances, ~1–10% of the total binding sites are strong metal binding sites. These minor sites are less in numbers; include all those which exhibit a wide range of binding energies including strongly complexing –N and –S containing sites.

The remaining binding sites, ~90–99%, are weak binding sites, which form weak complexes. The major sites are less diverse in type, but in number they represent the majority of the complexation sites. Carboxylic and phenolic-OH groups represent major sites in humic substances.

Pearson correlation analysis also suggests that both the metals formed stronger complexes with minor sites (mainly –S and –N containing group) of different well characterized humic substances. The major sites of the studied humic substances were found to form dynamic complexes with both the metals. This study indicates that Cu interacts more strongly with humic substances compared to Ni. It was found that both Ni and Cu had different affinities towards different humic substances from different origins. Both the metals underwent strong complexation reactions with NAHA as well as SAHA, compared to NAFA and SRNOM.

Speciation of Cu and Ni by DGT

Dynamic metal speciation data for Ni and Cu in the aqueous model solutions (by DGT) are presented in Table 4. Again, both metals had different affinities towards the different humic substances from the different origins. Table 4 shows that both Ni and Cu undergo strong complexation reactions with soil and aquatic humic acids (SAHA and NAHA). The interactions of Ni with NAFA and SRNOM were much weaker (as observed by the increasing DGT labile Ni concentrations in presence of NAFA and SRNOM). However, Cu interacted strongly with all the four humic substances used in this study. The DGT labile concentrations of Cu complexes in presence of SAHA and NAHA were small, and indicate

the strong interactions of Cu with both the aquatic and soil humic acids. The interactions of Cu with NAFA and SRNOM were much stronger than Ni under the same experimental conditions. It is known that humic acids contain more metal binding sites compared to fulvic acids and NOMs (CHEAM, 1973; Tokudome, S.; Kanno, 1965). In our study, SAHA was used as a representative soil humic acid. The kinetic speciation data also showed that Ni and Cu underwent strong complexation reaction with SAHA and NAHA compared to other aquatic humic substances. The analytical window of the DGT technique was changed by altering the thickness of the diffusive gel. Increasing lability of Ni-humic substances complexes with the increasing diffusive layer thicknesses indicates that labile complexes of Ni were formed with different binding sites with diverse binding energies in humic substances (Figure 3 a).

However, it is interesting to note that there was a small increase in lability of Cu complexes with the increasing diffusive layer thicknesses (Figure 3b), which indicates that Cu interacted very strongly with all four humic substances and formed inert complexes.

Approximately 35% of the total dissolved Ni-SAHA complexes dissociated within d_g^2/D_{Ni} s. (i.e., $(0.040+0.013)^2/5.77 \times 10^{-6} \approx 486$ s in the diffusive gel with thickness of 0.4mm. Approximately, 23%, 67% and 51% of Ni-NAHA, Ni-NAFA and Ni-SRNOM complexes dissociate within the same time in the diffusive gel, respectively. The strongest interaction was observed between Ni and NAHA, followed by SAHA, NAFA and SRNOM. However, further increases in the thickness of the diffusive gel (d_g) (from 0.4 mm to 0.8 mm to 1.6mm) increased the concentration (percentage) of the DGT labile fractions of Ni in the presence of all the different humic substances (Table 4). The maximum DGT labile fraction of Ni was found when the diffusive gel thickness was 1.6 mm (in all cases). The percentage of labile fraction Ni-SAHA complexes was found to be minimum compared to rest of the Ni-humics complexes. However, Ni-NAFA and Ni-SRNOM complexes were found to form comparatively weaker complexes. This observation suggests that interaction of Ni with SAHA is much stronger than the interaction of Ni with other humic substances used in this study under the existing experimental conditions. The change in DGT labile fraction of Ni suggest that the stability of Ni complexes follow the order of SAHA>NAHA>NAFA≈SRNOM.

It was found that small fractions of Cu-humics complexes were DGT labile. Approximately 4% of the total dissolved Cu-SAHA complexes dissociated within d_g^2/D_{Ni} s. (i.e., $(0.04+0.013)^2/6.06 \times 10^{-6} \approx 465$ s in the diffusive layer of 0.0.053 cm thickness. Approximately 3%, 8% and 11% of Cu-NAHA, Cu-

NAFA and Cu-SRNOM complexes dissociated within the same time in the diffusive gel. This observation suggests that Cu interacts very strongly with all four humic substances used in our study.

It is interesting to note that the increasing thickness of diffusive layer (d_g+d_f) (by changing the thicknesses of the diffusive gel from 0.4 mm to 0.8 mm to 1.6 mm) did not increase the concentration (percentage) of the DGT labile fractions of Cu in presence of all the different humic substances (Table 4). This further suggests that Cu interacts much more strongly with these humic substances than Ni. The percentage of labile fraction Cu-NAHA complexes was found to be minimal compared to rest of the Ni-humic substances complexes. However, Cu-NAFA and Cu-SRNOM complexes were found to form comparatively weaker complexes. This observation suggests that the interactions of Cu with SAHA and NAHA were stronger than the interactions of Cu with either NAFA or SRNOM. The change in DGT labile fraction of Cu suggests that the stability of Cu-complexes followed the order of NAHA \approx SAHA $>$ NAFA \approx SRNOM.

The CLE method was effective for the measurement of total labile (dynamic) metal complexes and their dissociation rate constants of both the metals (Cu and Ni) in a quasi labile system. The DGT technique was detecting less concentration of labile metals complexes in the same system. However, both techniques should have monitored the same concentrations of labile metal complexes (as Chelex-100 was the competing ligand and binding resin used in both techniques). The kinetics of dissociation of metal complexes was found to be fast (see kinetic speciation data in Table 2) and thus, one could easily understand that diffusion controlled metal flux (towards the binding resin in DGT) was responsible for the underestimation of labile metal complexes by DGT. These differences permitted us to estimate diffusion coefficients of the labile metal complexes in the quasi labile system.

Combination of DGT and CLE experiments to determine the diffusion coefficients of the labile complexes

The typical range in time required for both the metals to reach equilibrium in presence of different humic substances in CLE was $\sim 10^3$ - 10^4 s. The time required to attain a steady state profile in the DGT technique depends on the diffusive gel thickness. In the current study, the time required to attain a steady state profile with diffusive gel of 1.6mm was $\sim 10^3$ - 10^4 s for both the metals. Thus, the dynamic complexes obtained from the CLE technique were compared with the labile complexes obtained by the DGT technique when a diffusive gel of 1.6 mm thickness was used. This comparison is reasonable as the data obtained from both the techniques were within the same analytical windows.

The contribution of free metal and complexed metal to the total metal accumulated in the binding resin gel of DGT will be governed by the rates of diffusion of each metal species. Determination of the average diffusion coefficient of the metal complexes is possible if and only if the system is fully labile. The calculated diffusion coefficients of labile complexes of Ni and Cu in presence of different humic substances are presented in Table 5. The diffusion coefficients of labile Ni complexes were very similar to that of free Ni²⁺. This indicates that the labile Ni complexes in our experimental systems were small with very fast diffusion rate coefficients and may be a good representation of bioavailable Ni species. It has been reported previously that Ni usually prefers to form small complexes with humic substances (Hassan et al., 2006).

In contrast to Ni, Cu was associated with different molecular weight fraction of the humic substances (Table 6)(Balistreri 2012; Beckett 1987; Hayes 1989; Pinheiro 1998; Lead 2000; Lead 1999; Pinheiro 1996; Reid 1991). The average diffusion coefficients of labile Cu-humate complexes were slow, which indicated that Cu formed complexes with high molecular weight fractions of humic substances. However, the complexes of Cu with SRNOM were found to have relatively fast diffusion coefficients. This finding suggests that Cu interacts with both larger and smaller humic substances. Similar observations have already been reported in literature (Chakraborty 2007).

The values of diffusion coefficients of labile Ni and Cu complexes that were measured in this study are in good agreement with the limited literature that is available. Table 6 indicates that there are differences among the diffusion coefficients of Cu-HA, Cu-FA and Cu-NOM complexes. The diffusion coefficients of the Cu-humate complexes are consistently lower (larger size) than those of the Cu-fulvate and NOM (Table 6), which is probably due to their greater hydrophobicity and larger molar mass. The similarity of the diffusion coefficient of Cu-complexes to that of HA, FA or NOM indicates that labile Cu complexes were associated with HA, FA or NOM. It is interesting to note that the diffusion coefficients of Ni complexes were higher than the diffusion coefficients of HA, FA, NOM and Cu complexes. This indicates that the labile Ni complexes were likely small and probably not associated with HA, FA or NOM.

Conclusion

The combination of these two techniques was found to be very useful in determining diffusion coefficients of labile metal- humic substances complexes in quasi-labile systems. The values of diffusion coefficients of labile Ni and Cu complexes determined in this study are in good agreement with a limited

set of results from the literature. This finding is novel and can be very useful in further improving our understanding of the metal–humic substances interactions in natural environments. One of the major advantages of this approach (of combining CLE and DGT) is that it provides diffusion coefficients of labile complexes directly in a quasi labile system.

Acknowledgements

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Table 1: The elemental compositions, concentrations of carboxylic and phenolic–OH and carbohydrate in Nordic aquatic humic acid (NAHA), Nordic aquatic fulvic acid (NAFA) and Suwannee River NOM (SRNOM) reported by International humic substance society (IHSS)

Standard HA Cat. No.	Nordic Lake 1R105H	Nordic Lake 1R105F	Suwannee River 1R101N	SAHA
H₂O	9.1	9.2	8.15	
Ash	0.31	0.45	7	
C	53.33	52.31	52.47	48.36
H	3.97	3.98	4.19	4.24
O	43.09	45.12	42.69	26.91
N	1.16	0.68	1.1	0.78
S	0.58	0.46	0.65	0.78
P	0.01	<0.01	0.02	
δ¹³C	-27.8	-27.8	Nd	
δ¹⁵N	-1.55	-3.19	Nd	
Carboxylic	9.06	11.16	9.85	3.32
Phenolic	3.23	3.18	3.94	
Carbohydrate	113.3	52.1	nd	

Table 2: kinetically distinguishable components and their associated dissociation rate constants.

$[\text{Cu}]_T$	Humics (5.0 mg.dm ⁻³)	$c_1(\%)$	$k_{d1} \times 10^3 (\text{s}^{-1})$	$c_2(\%)$	$k_{d2} (\text{s}^{-1})$
$5 \times 10^{-7} \text{ M}$	SAHA	40.5 ± 0.8	2.6 ± 0.1	59.5 ± 1.2	$>1 \times 10^{-6}$
	NAHA	29.5 ± 0.4	4.9 ± 0.8	70.5 ± 0.9	3.2×10^{-5}
	NAFA	62.5 ± 1.3	0.9 ± 0.2	37.5 ± 0.8	3.3×10^{-5}
	SRNOM	81.7 ± 4.9	3.1 ± 0.4	18.3 ± 1.1	1.5×10^{-4}
$[\text{Ni}]_T$	Humics (5.0 mg.L ⁻¹)	$c_1(\%)$	$k_{d1} \times 10^3 (\text{s}^{-1})$	$c_2(\%)$	$k_{d2} (\text{s}^{-1})$
$5 \times 10^{-7} \text{ M}$	SAHA	51.7 ± 1.0	5.0 ± 0.6	48.4 ± 3.4	$>1 \times 10^{-6}$
	NAHA	34.4 ± 0.4	9.3 ± 0.8	65.7 ± 3.8	1.8×10^{-4}
	NAFA	69.9 ± 1.5	5.2 ± 0.6	30.1 ± 2.1	2.9×10^{-4}
	SRNOM	62.5 ± 3.8	2.9 ± 0.4	37.5 ± 1.4	3.3×10^{-4}

SAHA- soil humic acid from Sigma Aldrich; NAHA- Nordic aquatic humic acid ; NAFA- Nordic aquatic fulvic acid ; SRNOM- Suwanee River natural organic matter

All the speciation parameters are reported as their mean \pm standard deviation

Table 3: The Pearson correlation matrix between the dynamic and inert complexes of Ni and Cu with the – S, -N, -COOH, Phenolic-OH concentrations of the humics

<i>Ni</i>	<i>N</i>	<i>S</i>	<i>Carboxylic</i>	<i>Phenolic</i>	<i>Average</i>	<i>Dynamic</i>	<i>Inert</i>
N	1						
S	0.883376	1					
Carboxylic	-0.96466	-0.72867	1				
Phenolic	0.450641	0.816465	-0.1995	1			
Average	-0.78378	-0.40131	0.91972	0.201201	1		
Dynamic	-0.74292	-0.34256	0.893042	0.262767	0.997997	1	
Inert	0.742921	0.342565	-0.89304	-0.26277	-0.998	-1	1

Cu	N	S	Carboxylic	Phenolic	Average	Dynamic	Inert
N	1						
S	0.883376	1					
Carboxylic	-0.96466	-0.72867	1				
Phenolic	0.450641	0.816465	-0.1995	1			
Average	-0.78378	-0.40131	0.91972	0.201201	1		
Dynamic	-0.26328	0.219551	0.508173	0.742563	0.805484	1	
Inert	0.263284	-0.21955	-0.50817	-0.74256	-0.80548	-1	1

Table 4: Percentage of labile metal (nickel and copper) complexes in the aqueous model solutions by using DGT

[M] _T	Humics (5.0 mg.L ⁻¹)	DGT labile Ni complexes			DGT labile Cu complexes		
		0.4mm	0.8mm	1.6mm	0.4mm	0.8mm	1.6mm
5×10 ⁻⁷ M	SAHA	34.7 ± 1.2	49.5 ± 2.1	53.3 ± 3.3	3.7 ± 0.5	4.3 ± 0.3	5.7 ± 0.6

	NAHA	23.1 ± 2.2	43.1 ± 4.3	74.9 ± 3.2	2.9 ± 0.3	2.9 ± 0.2	3.0 ± 0.3
	NAFA	66.5 ± 4.8	84.0 ± 6.3	86.0 ± 4.3	7.6 ± 1.3	9.6 ± 0.3	13.2 ± 1.3
	SRNOM	51.3 ± 2.7	82.8 ± 7.1	86.6 ± 8.2	10.9 ± 1.0	11.1 ± 1.3	16.9 ± 6.2

SAHA- soil humic acid from Sigma Aldrich; NAHA- Nordic aquatic humic acid ; NAFA- Nordic aquatic fulvic acid ; SRNOM- Suwanee River natural organic matter

All the speciation parameters are reported as their mean ± standard deviation

Table 5: Calculated diffusion coefficients of labile complexes of Ni and Cu in presence of different humic substances

Cu	DGT Dyn(%)	CLE dyn (%)	$D_M(\text{cm}^2 \cdot \text{s}^{-1})$	$D_{ML}(\text{cm}^2 \cdot \text{s}^{-1})$	Ni	DGT Dyn(%)	CLE dyn (%)	$D_M(\text{cm}^2 \cdot \text{s}^{-1})$	$D_{ML}(\text{cm}^2 \cdot \text{s}^{-1})$
SAHA	5.7	40.5	6.06×10^{-06}	8.5×10^{-07}	SAHA	52.3	51.7	5.77×10^{-06}	5.6×10^{-06}
NAHA	3.0	29.5	6.06×10^{-06}	6.2×10^{-07}	NAHA	74.9	100	5.77×10^{-06}	4.3×10^{-06}
NAFA	13.2	100	6.06×10^{-06}	8.0×10^{-07}	NAFA	86.0	100	5.77×10^{-06}	4.9×10^{-06}
SRNOM	76.9	100	6.06×10^{-06}	6.7×10^{-06}	SRNOM	86.6	100	5.77×10^{-06}	5.0×10^{-06}

SAHA- soil humic acid from Sigma Aldrich; NAHA- Nordic aquatic humic acid ; NAFA- Nordic aquatic fulvic acid ; SRNOM- Suwanee River natural organic matter

Table 6. Comparison of diffusion Coefficients of HSs with the diffusion coefficients data from literature

NOM	Technique	Diffusion coefficient of M-complexes ($\text{cm}^2 \cdot \text{s}^{-1}$) $\times 10^6$	References	Diffusion coefficient of M complexes (This study)		
				($\text{cm}^2 \cdot \text{s}^{-1}$) $\times 10^6$		
				Cu complexes	Ni complexes	
Peat and terrestrial NOM	SAHA	Chromatography	0.2-0.9	Reid 1991		
	Soil HA	AUC	0.05-0.4	Hayes 1989	0.85	5.6
	Peat HA	PCS	0.05-0.2	Van Leeuwen 1998		
	SRFA	FCS/PFG-NMR/FIFFF	1.9-3.5	Beckett 2000	0.8	4.9
Aquatic NOM	HA/FA	Voltammetry	1.0-4.7	Greter 1979		
	FA	Voltammetry	0.6-1.2	Pinheiro 1996	0.8	4.9
	NOM	FCS	2.1-2.9	Lead 1999	4.7	5.0
	HA	DGT	0.67	Balistreri 2012	0.62	4.3

Flow Field-Flow Fractionation (FIFFF); Fluorescence Correlation Spectroscopy (FCS); Pulsed Field Gradient-NMR (PFG-NMR); AUC - analytical ultracentrifugation; PCS - photon correlation spectroscopy.

SAHA- soil humic acid from Sigma Aldrich; NAHA- Nordic aquatic humic acid ; NAFA- Nordic aquatic fulvic acid ; SRNOM- Suwanee River natural organic matter; HA-Humic Acid, FA-Fulvic Acid, NOM-Natural Organic Matter

CAPTIONS AND LEGENDS FOR FIGURES

Figure 1: *The concentrations profiles of dissolved M-HS complexes with respect to time in a) fully-labile, b) inert and c) Quasi-labile system (during CLE method) and DGT steady-state concentrations profiles for the coupled diffusion of M and ML; a) fully-labile, b)inert and c)Quasi-labile system*

Figure 2: *Kinetic extraction of (a) Ni and (b) Cu in presence of four different types of HSs from different origins(SAHA- soil humic acid from Sigma Aldrich; NAHA- Nordic aquatic humic acid ; NAFA- Nordic aquatic fulvic acid ; SRNOM- Suwanee River natural organic matter)*

Figure 3: *Change in labile fractions of Ni complexes (a) and Cu complexes (b) with humic substances from different origins (SAHA- soil humic acid from Sigma Aldrich; NAHA- Nordic aquatic humic acid ; NAFA- Nordic aquatic fulvic acid ; SRNOM- Suwanee River natural organic matter) with the increasing diffusive gel thicknesses in DGT*

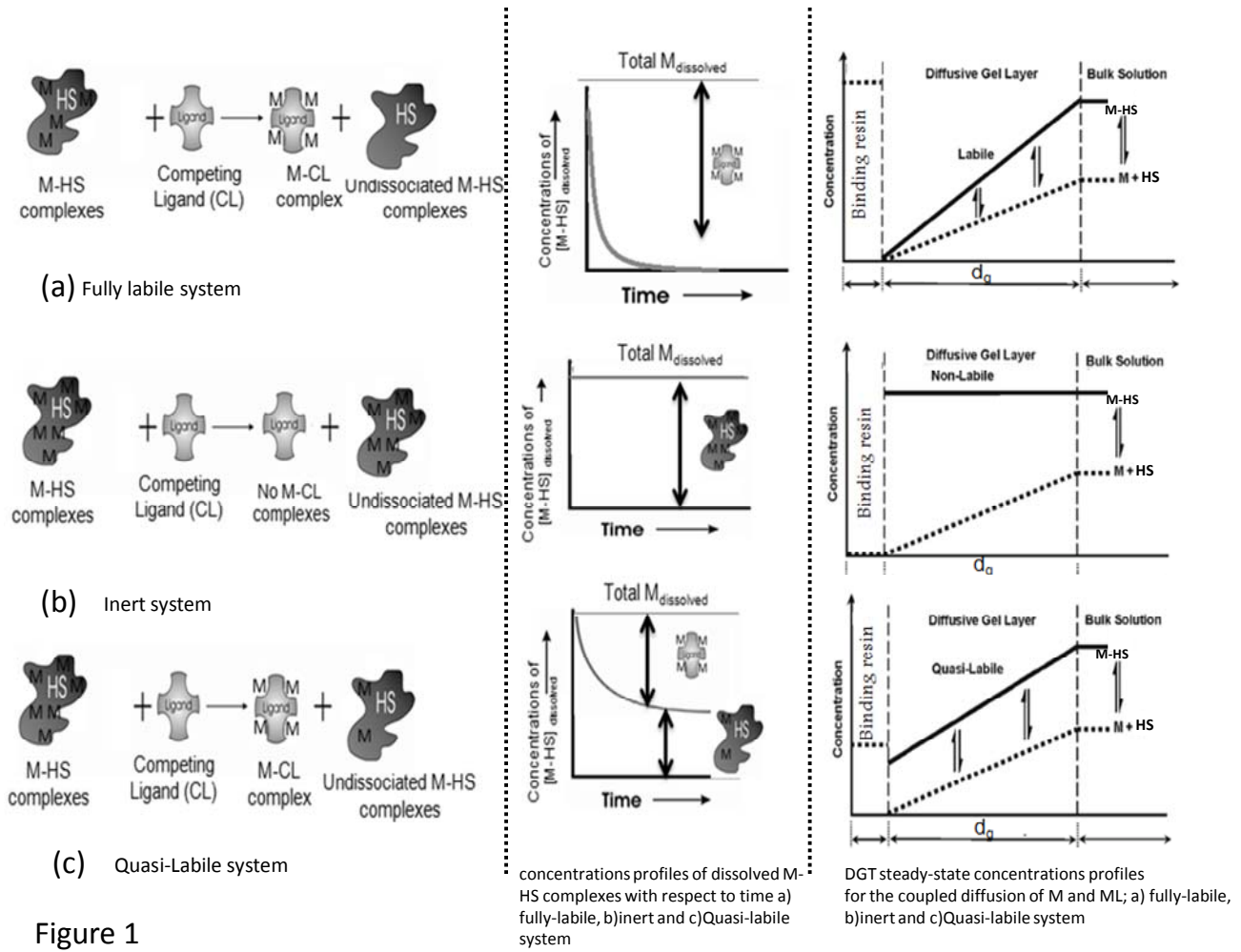


Figure 1

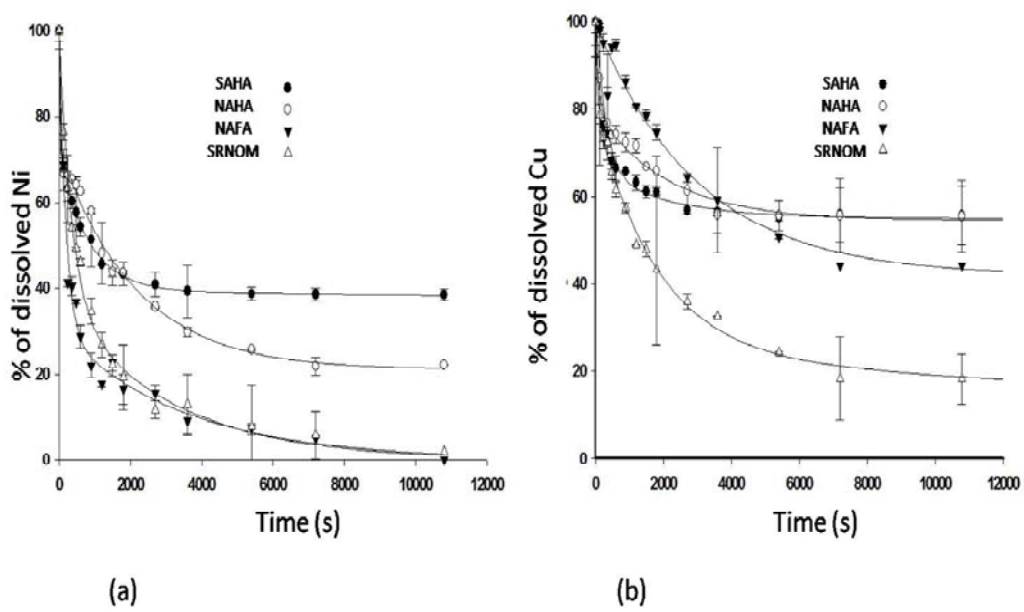


Figure 2

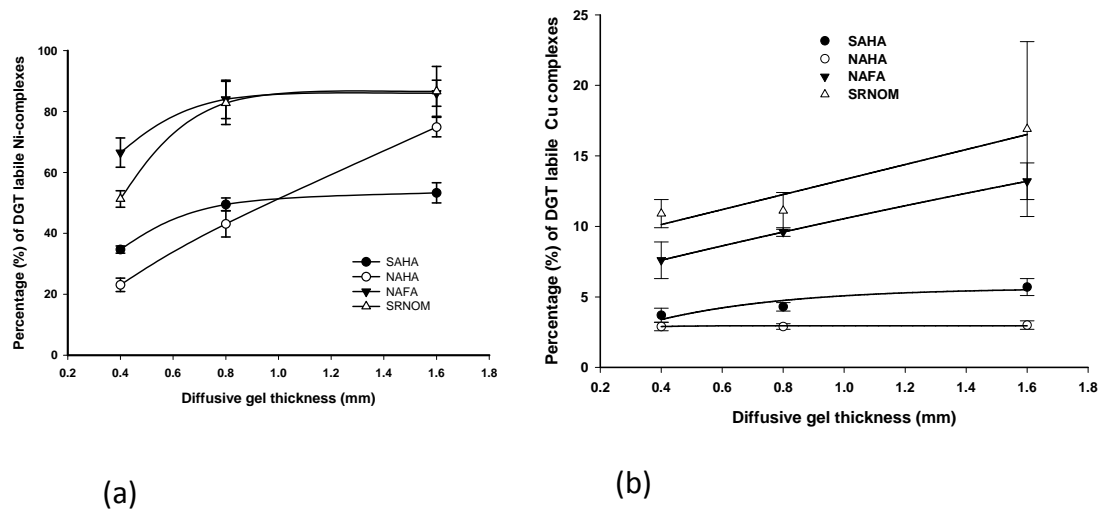


Figure 3