

Dynamic partitioning
of nanoparticulate metal species
between gel layers and aqueous media

Pascal L.R. van der Veeke

Thesis committee:

Thesis supervisor:

Prof. dr. M.A. Cohen Stuart
Professor of Physical Chemistry and Colloid Science
Wageningen University

Thesis co-supervisor:

Prof. dr. H.P. van Leeuwen
Associate professor, Laboratory of Physical Chemistry and Colloid Science
Wageningen University
and extraordinary professor, Environmental Physical Chemistry
University of Geneva, Switzerland

Other members:

Prof. Dr. W.H. van Riemsdijk, Wageningen University
Prof. Dr. W. Davison, Lancaster University, U.K.
Dr. ir. J.P. Pinheiro, University of the Algarve, Portugal
Dr. ir. R.F.M.J. Cleven, RIVM, Bilthoven

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Dynamic partitioning of nanoparticulate metal species between gel layers and aqueous media

Pascal L.R. van der Veeken

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Pascal L.R. van der Veeken

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Chapter 1

General introduction

1. General introduction

1.1. Motivation

Over the 20th century, man began to realize the downsides to the industrial revolution. Major concerns arose regarding the accumulation of industrial pollutants, including heavy metals, in the environment (1, 2). Investigations on the toxicological effects of metals and other compounds in soils and waters became a serious matter (3-5). These developments generated the need for suitable methods of measuring the reactive forms of metals in environmental media. Considering the tremendous diversity of many of the chemical compounds involved, this is not an easy task. The physicochemical forms of metals in the environment include completely hydrated "free" ions like $\text{Pb}(\text{H}_2\text{O})_6^{2+}$, simple inorganic complexes, metals bound to macromolecules and metals adsorbed to suspended matter (6). The determination of the concentrations of these different physicochemical species is called *speciation* analysis. If the analysis includes the reactivity of the species (i.e. how fast species react to changes in the system, e.g. addition of a reactant, dilution, salinity or even the introduction of a measurement device), then it is called *dynamic* speciation analysis. Speciation of a metal is also a major factor in its availability towards organisms, i.e. *bioavailability*. It is the rule, rather than the exception, that the toxicity of a metal is not directly linked with its total concentration, but rather with the concentration of one particular type of species or group of species. Bioavailability assessments therefore require speciation analysis.

Many methods of determining species distribution are available, and they potentially offer ways to collect entire spectra of species properties. In general, many of the methods of determining a species concentration include some pretreatment of samples, and this can unintentionally affect the speciation in the sample (7-9). In trying to determine the original speciation in environmental samples, passive samplers (meaning they are based on unforced transport of chemical species from a sample medium to a collector) have been introduced. This thesis is concerned with two particular methods that use passive diffusion into a gel layer to obtain information on the speciation and lability of a metal complex system. They are known as Diffusive Equilibration in Thin film (DET) and Diffusive Gradient in Thin film (DGT).

1.2. Lability of species

In the field of non-equilibrium speciation sensors, an important concept is *lability*. Roughly speaking, lability is a measure of how well a complexation reaction can maintain equilibrium under conditions where one of the species is involved in an interfacial conversion process (10). For many sensor devices and bioaccumulation processes the crucial conversion takes place at an interface, e.g. in the form of adsorption, transfer to the other phase, chemical reaction, etc. Since generally it is the free metal that is available for uptake by organisms, the lability of a metal complex species is related to its reactivity with respect to dissociation into free metal. As a consequence, there is a significant relation between the overall bioavailability of a metal and the labilities of its various complex species. Since the term "lability" is relevant throughout many issues on dynamic metal speciation, we here take a closer look at some details of the concept.

1.2.1. Complexation

Let us consider a simple system in which a metal ion M (short notation for $M(H_2O)_6^{n+}$) can form a complex with a ligand L:



The equilibrium constant for this reaction is usually denoted as the stability constant K ($= c_{ML}/c_M c_L$) which equals k_a/k_d (the rate constants for association and dissociation of the complex, respectively). In the frequently encountered case of a sufficient excess of ligand, the product of k_a and the ligand concentration $k_a c_L$ (k'_a in short) is approximately constant, and the association of M with L is quasi-monomolecular. The characteristic lifetimes of the individual species M and ML are then $1/k'_a$ and $1/k_d$, respectively, and k'_a/k_d (usually denoted as K') equals the ratio c_{ML}/c_M . The case where the rate constants are very low compared to the relevant timescale t ($k'_a t, k_d t \ll 1$) is trivial; equilibrium is frozen and the complex species is effectively *inert*. The opposite situation, where the rate constants are very high ($k'_a t, k_d t \gg 1$), is denoted as *dynamic*. This means that the volume complexation reaction in eq 1 maintains equilibrium on the experimental timescale t , implying that each individual metal ion frequently switches from M to ML and back.

1.2.2. Reaction layer concept

In the case of an interfacial reaction of the metal, the rate is usually represented in terms of the corresponding flux J . The nature of J , as measured by a speciation sensor, depends on the specific parameters of the system which include the association/dissociation rate constants of the various metal species and the mobilities of all species involved in the process of diffusion of metal to the interface. Adjacent to the reactive interface, there will be a layer of solution where the equilibrium between the surface-reactive metal species, usually the free M and the surface-nonreactive ML is distorted. The thickness μ of this so-called reaction layer derives from the diffusional displacement of M (11) before it re-associates with the ligand L (12). Thus, the formulation of μ is based on two basic properties of the free metal ion, i.e. its mobility (diffusion coefficient D_M) and its lifetime ($1/k'_a$):

$$\mu = (D_M / k'_a)^{1/2} \quad (2)$$

Figure 1 illustrates the meaning of μ in terms of the concentration profiles of M and ML near an interface where the species M is involved in a reaction. The pertaining flux due to dissociation of ML, J_{kin} , then simply is the product of $k_d c_{\text{ML}}$, the volume dissociation rate, and the thickness of the reaction layer μ :

$$J_{\text{kin}} = k_d c_{\text{ML}} \mu \quad (3)$$

For the two limiting cases of inert and labile complexes, the resulting maximum metal flux J^* for ($c_M(0) = 0$) can be derived from the general expression for J^* (13). For an inert complex, J_{kin} is essentially zero and the flux is simply

$$J^* = \frac{D_M c_M^*}{\delta} \quad (4)$$

illustrating that the flux is solely determined by the free metal.

In the other limit of a fully labile system and for the case where M and ML have different diffusion coefficients, the general expression for J^* comes to

$$J^* = \frac{\bar{D} c_T^*}{\delta} \quad (5)$$

where $c_T^* = c_M^* + c_{\text{ML}}^*$ and \bar{D} is the mean diffusion coefficient of M and ML defined as:

$$\bar{D} = \frac{D_M c_M + D_{\text{ML}} c_{\text{ML}}}{c_T} \quad (6)$$

Eq 5 expresses that the kinetic process of dissociation of ML is so fast that the flux is limited by the coupled diffusion of M and ML. In other words, for a labile system the kinetic flux J_{kin} is much larger than the diffusion controlled flux and therefore the latter is the controlling one. In the concentration profile this situation corresponds to μ becoming so small as to be negligible compared to δ .

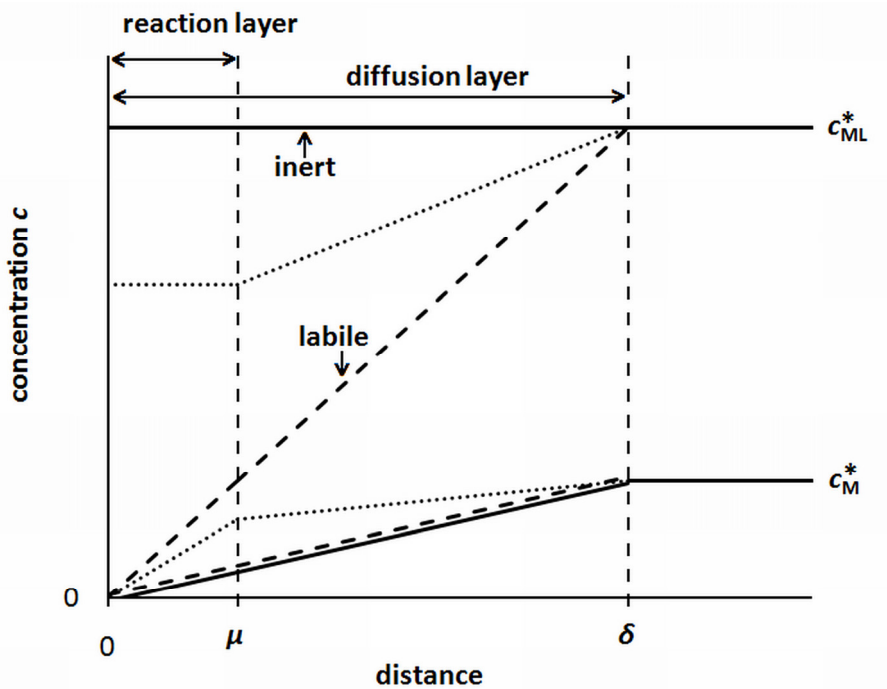


Figure 1: Schematic illustration of the reaction layer (thickness μ) and the diffusion layer (thickness δ) in relation to the concentration profiles of free metal M and complex ML, for the case of a dynamic system with $D_M = D_{ML}$. For simplicity the surface is considered as a perfect sink for M ($c_M = 0$). The inert complex case is given by (—), whereas the fully labile system, with $\mu/\delta \rightarrow 0$, is denoted by (---). The case of kinetic limitation of the contribution of ML to the overall metal flux (dotted lines) is intermediate between the inert and labile cases (not discussed in detail).

In case of a mixture of different complex species, with different degrees of lability, the kinetic contribution to the flux is governed by the simultaneous dissociation of the different complexes, collectively linked to the diffusion profile of M (14). With decreasing rates of diffusion (longer time scale, larger δ), complex species become more labile. For larger species with inherently lower diffusion coefficients, there is the complication that the diffusion layer thickness δ is a function of D . As a consequence, for a given metal δ varies with the speciation. One particular technique that avoids this complication by independently controlling the diffusion layer thickness is DGT.

1.3. DET/DGT

DET and DGT (15–17) are speciation techniques based on metal permeation into a gel layer. In DET (18, 19), a gel layer is kept in contact with the sample solution until partition equilibrium is achieved. The final total metal content of the gel reflects the physical ability of species to enter the gel phase, and for that size is the primary controlling parameter. Because of the equilibrium nature, lability is immaterial in DET.

DGT, on the other hand, effectively employs its gel layer as a well-defined diffusion layer between the sample solution and a strongly complexing resin. A schematic representation of a DGT unit is shown in Figure 2, from a design by Zhang et al. (20). The hydrogel is covered only by a protective membrane filter (0.45 μm pore size). The gel itself allows ions and relatively small complexes to pass through to the ion-exchange resin layer, which acts as a perfect metal sink and is otherwise completely separated from the bulk solution.

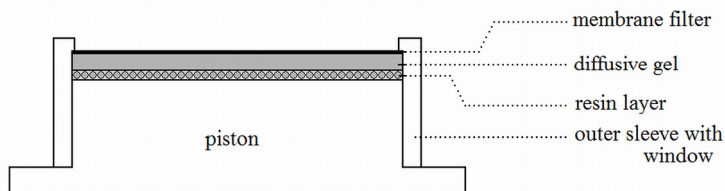


Figure 2. Sketch of a regular DGT deployment unit. The unit is designed such that the target metal species can only reach the resin layer by diffusion through the gel layer. The effective contact area between DGT sensor and sample solution is usually on the order of a few cm^2 .

In DGT, the thickness of the diffusive gel layer (δ_g) is chosen well above δ_{sample} , i.e. order 10^{-3} m, and under such conditions it is assumed that the contribution of non-penetrating complexes to the steady-state flux can be ignored. According to Fick's first law, the steady-state flux (J) of a type of metal species through the gel layer then depends on its diffusion coefficient (D_g), the diffusion distance (δ_g), and the difference between the concentration in solution (c_w^*) and that at the gel/resin interface (c'):

$$J = \frac{D_g (c_w^* - c')}{\delta_g} \quad (7)$$

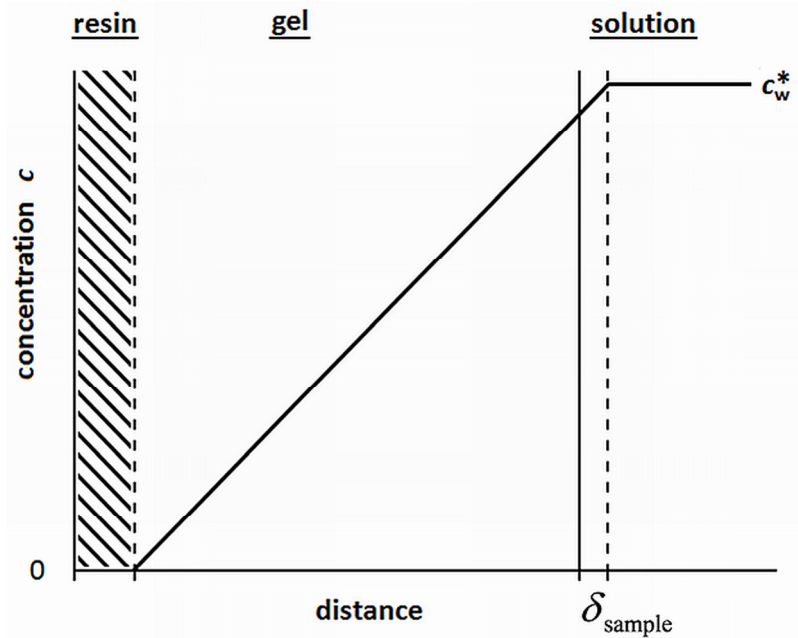


Figure 3. Steady state flux concentration profile of a single metal species M . Ideally, the resin acts as a perfect sink, so that at the resin/gel interface $c=0$. The thickness of the gel layer δ_g is usually large enough to allow for the approximation that c at the gel/sample interface equals c_w^* .

For a sufficiently strong ion-exchange resin with a large excess capacity, c' becomes essentially zero, and eq 2 reduces to

$$J = \frac{D_g c_w^*}{\delta_g} \quad (8)$$

This situation is illustrated in figure 3.

Before achieving steady-state flux conditions, the system first passes through a transient stage. The leading time constant τ_{ss} for this transient process is given by

$$\tau_{ss} = \delta_g^2 / D_g \quad (9)$$

In order to eliminate the impact of the initial transient stage and to make equations on the level of eq 8 applicable, the DGT deployment time needs to be sufficiently long compared to τ_{ss} .

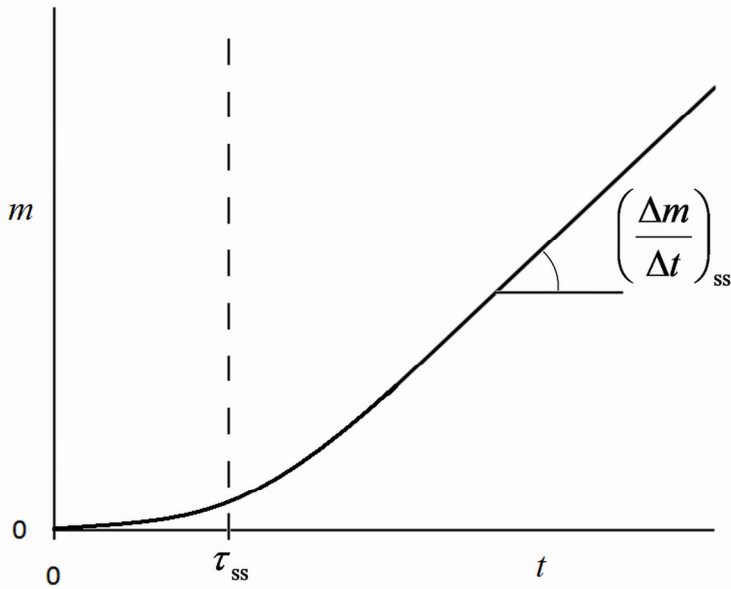


Figure 4. Sketch of the temporal accumulation of target metal, in terms of its mass m , in the backing resin of a DGT sensor. Starting with a metal-free sensor unit, the flux J passes through a transient stage, before ultimately reaching steady-state with a constant $(\Delta m / \Delta t)$.

Once a steady-state has been achieved, the metal transport across the gel is described by eq 4. Rewritten in practical quantities it reads

$$\left(\frac{\Delta m}{\Delta t}\right)_{ss} = \frac{D_g A c_w^*}{\delta_g} \quad (10)$$

where (Δm) is the total mass of metal transported towards the resin layer during time interval (Δt) , for an effective sensor surface area A . The accumulated metal is then used to calculate the concentration in solution. For a sample solution containing various metal species, the result is the sum of the free and DGT-labile metal species.

DGT has proved to be a successful means for in-situ measurement of the concentration of free metal and labile complexes in natural waters (21, 22). When combining DGT deployment with separate DET measurements, equilibrium partition characteristics can be taken as the starting point in the analysis of DGT fluxes (23). Such a combination of equilibrium and flux techniques is especially useful in cases where the partition of the relevant species between the sample solution and the gel phase is not trivial.

1.4. Outline of this thesis

This thesis comprises an investigation on the application of DET and DGT to the dynamic speciation analysis of metal complex systems, involving nanoparticulate and colloidal ligands. *Chapter 2* considers the permeability of the DET/DGT hydrogels towards various ligand species. Although assumptions on the size of the pores in the gel have been made in the past, the high water content of hydrogels makes clear-cut determination of the pore size characteristics practically impossible. Here we apply hard-sphere latex particles as permeability probes, and discuss what effect the permeation of larger ligands may have on DET/DGT measurements. *Chapter 3* discusses the possible effects of the structural electric charge inherent to the chemical nature of the polyacrylamide hydrogel. Especially in the lower ionic strength regime, Donnan partitioning is significant and concentrations of charged species have to be corrected.

Chapters 4 to 6 are concerned with the effects of natural organic matter on DET/DGT metal speciation analysis. Two test compounds are used, fulvic acid (FA) and humic acid (HA), which in natural soils and waters generally are of an extremely heterogeneous nature. Whilst some effects of humic and fulvic acids on the DGT response of metal species have been observed before, the gel permeation properties of the actual humic and fulvic acid compounds themselves have not been analyzed thus far. In order to fill this gap, *Chapter 4* studies the permeation of FA and HA and the possible interaction with the hydrogel backbone. DET is used to determine whether these natural complexing

agents enter the gel layer, and if they might even accumulate in the gel. *Chapter 5* analyzes the extent of humic matter accumulation in the gel phase for a sample medium containing both free humics and humic metal complexes, under various conditions of ionic strength and total humic concentrations. Special attention will be paid to the role of the metal ion in this accumulation and the ensuing impact on metal partitioning between sample and gel. *Chapter 6* explores the impact of accumulation of humic acid in the gel layer on the properties of the DGT metal flux, for various experimental conditions. The different effects of humic matter will be discussed in terms of the reliability of DGT data and the ramifications for interpretation of measurements on samples containing heterogeneous and uncharacterized humic matter.

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Chapter 2

Metal speciation by DGT/DET in colloidal complex systems

Pascal L.R. van der Veeken, José P. Pinheiro, Herman P. van Leeuwen

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Abstract

Gel layer based sensors are increasingly employed for dynamic trace metal speciation analysis in aquatic media. In DGT (Diffusive Gradient in Thin Film), two different types of polyacrylamide hydrogels, designated as “open pore” and “restricted”, are commonly used for the diffusive gel layer. Whilst both gels are known to be fully permeable to metal ions and small complexes, colloidal particles with radii from tens to hundreds of nm have generally been assumed to be excluded from the gel. Here we show, however, that for dispersions with Pb(II) as the probe metal and monodisperse latex particles as metal-binding agents, relatively large particles do enter the gel to a significant extent. By complementing DGT flux analysis with DET accumulation data for the colloidal complex systems, it is demonstrated that with radii up to some 130 nm permeation for particles is substantial. The consequences for interpretation of dynamic speciation data furnished by gel-based sensors are discussed.

2.1. Introduction

Dynamic speciation is an important aspect of modern aquatic metal ion research (1-5). The different techniques available for metal speciation analysis use a variety of species selection principles and effective measurement timescales (6, 7). Diffusive Gradients in Thin Film (DGT) and Diffusive Equilibration in Thin Film (DET) (8-10) are speciation techniques based on metal permeation into a gel layer. The characteristics of the penetration of the gel by these species are closely linked with the pore size distribution of the gel, which thus far has not been quantitatively defined. For samples with polymeric and/or colloidal complexes, uncertainty exists as to the size range of complex species that may enter the gel.

In DET, the gel layer is kept in contact with the sample solution until partition equilibrium is achieved. The final total metal content of the gel phase reflects the physical ability of species to enter the gel, so size is the controlling parameter in this technique. DGT, on the other hand, effectively employs its gel layer as a diffusion layer between the sample solution and a strongly complexing resin, which acts as a perfect metal sink. After a limited transient stage, it measures the steady-state flux of the sufficiently labile species through the gel layer. The corresponding metal accumulation in the resin layer represents the integral of the flux over the deployment time. As with DET, the possible penetration by large species depends on the effective pore size of the diffusion gel layer but, contrary to DET, the DGT signal also depends on the lability and the mobility of the species inside the gel layer. Thus far, the pores in the diffusive gel have been assumed not to exceed 10 nm (11), but they have not been directly measured.

Two different types of gel are commonly used in DGT. They are generally referred to as the “open pore gel” and the “restricted gel”; the latter is expected to have the smaller pore sizes (11, 12). Diffusion coefficients for free metal ions in open pore gels and restricted gels are about 90 and 60% of their value in water, respectively (13). Diffusion cell experiments for several different complexes have also been performed on these gels (13). Specific structural properties of the polyacrylamide-based gels, as relevant for the pore size distributions, have remained largely unexplored (11). Only very recently agarose-type of gels were subjected to exploratory investigation (14-16).

The combination of DET equilibrium information and DGT dynamic information is particularly attractive for characterizing metal speciation in the case of relatively large metal species with low and/or poorly defined mobilities and labilities. Due to the modest contribution of such species to the steady-state DGT flux, their presence in the gel is much better quantified by DET. Thus we apply both DGT and DET to Pb(II) speciation in dispersions of spherical carboxylated latex particles with different sizes (i.e. well-defined mobilities). This will enable us to define the permeation of the gel by the labile

Pb(II)/particle complex and to provide a basis for DGT speciation analysis of samples containing colloidal metal species, as typically encountered in natural waters.

2.2. Theory

2.1.1. DET

In DET (17, 18) a gel layer is brought into contact with the sample solution until solute partition equilibrium establishes itself between gel and sample. The total metal concentration in the gel, c_{Tg}^* , is essentially equal to the sample concentration of the metal species if they can freely enter the gel, provided specific binding to the gel (19, 20) and Donnan effects (21, 22) are insignificant, and excluded volume in the gel layer is properly accounted for.

The DET equilibration process is generally diffusion-controlled. The usual conditions include that

- the initial concentration in the gel phase is zero,
- transfer of penetrating species across the water/gel interface does not involve any kinetic limitation,
- the ratio c_{ig}^*/c_i^* for penetrating species is unity (see restrictions above),
- the steady-state diffusion layer in solution is thin compared to the gel layer,
- the solution volume is much larger than the gel layer volume so that c_i^* remains constant.

According to Crank (23), the dependence of \bar{c}_g on time is then given by

$$\frac{\bar{c}_g(t)}{c_g^*} = 1 - \sum_{i=0}^{\infty} \frac{8}{(2i+1)^2 \pi^2} \exp\left[-(i+1/2)^2 \pi^2 D_g t / \delta_g^2\right] \quad (1)$$

where $\bar{c}_g(t)$ denotes the average concentration in the gel layer at time t and c_g^* the eventual equilibrium concentration. For $t \gg 4\delta_g^2 / \pi^2 D_g$, e.g. t well above 10^2 s for $D_g = 10^{-9} \text{ m}^2\text{s}^{-1}$ and $\delta_g = 0.5 \text{ mm}$, it suffices to take only the first term in the series. Eq 1 then reduces to

$$\frac{\bar{c}_g(t)}{c_g^*} = 1 - \frac{8}{\pi^2} \exp(-\pi^2 D_g t / 4\delta_g^2) \quad (2)$$

where $\pi^2 D_g / 4\delta_g^2$ is the characteristic DET equilibration rate constant (k). For instance, for a gel layer with 0.5 mm thickness and colloidal metal species 50 times larger than the free ion, this rate constant would come to approximately $2 \times 10^{-4} \text{ s}^{-1}$ (cf. 10^{-2} s^{-1} for the free metal). Similar time constants recur in DGT where the slower species determine the time necessary to establish steady-state diffusion.

2.2.2. DGT

Usually, the thickness of the gel layer is chosen sufficiently large (order 10^{-3} m) to render the diffusion in solution (4) insignificant. According to Fick's first law, the steady-state flux (J) of a metal species through the gel layer then depends on its gel diffusion coefficient (D_g), the diffusion distance (δ_g) and the difference between the concentration in solution (c^*) and that at the gel/resin interface (c'):

$$J = \frac{D_g (c^* - c')}{\delta_g} \quad (3)$$

where excluded volume effects are ignored. For a sufficiently strong ion-exchange resin with a large excess capacity, c' is essentially zero, and eq 3 reduces to

$$J = \frac{D_g c^*}{\delta_g} \quad (4)$$

so that the increase in mass of bound metal (Δm) with an increase in deployment time (Δt), equals

$$\Delta m = J A_g \Delta t \quad (5)$$

where A_g is the surface area of the gel/sample interface. The deployment time needs to be sufficiently long compared to the characteristic steady-state achievement time δ_g^2 / D_g , in order to render the impact of the initial transient on the slope $\Delta m / \Delta t$ insignificant.

2.2.3. Combining DGT and DET

When performing both DGT and DET on the same sample, it is possible to exploit the complementarity of the signals of the two techniques. Let us consider a dispersion containing only free metal and one type of labile colloidal complex under excess ligand conditions. The mean diffusion coefficient (\bar{D}) of such a system is a mobility-weighted average over the free metal M, and the particle-bound metal Mp (24):

$$\bar{D} = \frac{(D_M c_M + D_p c_{Mp})}{c_T} = D_M c_M \frac{(1 + \varepsilon K')}{c_T} \quad (6)$$

in which

- D_M and D_p are the diffusion coefficients for the free and the bound metal in the gel respectively (the particle is assumed to be sufficiently large so that $D_{Mp} \approx D_p$),
- c_T is $(c_M + c_{Mp})$ in which c_{Mp} is the equivalent volume concentration of the particle-bound metal. For a solid particle and M bound to surface sites, c_{Mp} is related to the surface concentration Γ_M via the total particle surface area A for a given dispersion with volume V :

$$c_{Mp} = \Gamma_M A / V \quad (7)$$

- ε equals D_p / D_M and K' is c_{Mp} / c_M . Note that K' is a constant only for essentially constant ligand concentrations (i.e. sufficient excess of surface ligand). For a given ion/particle combination, the relative importance of the two diffusion terms in eq 6 is determined by c_{Mp} / c_M . Only if this ratio between bound and free metal concentration ratio is well above D_M / D_p (which is already well above unity itself), will \bar{D} approach D_p . \bar{D} in the gel, \bar{D}_g , increases with decreasing fraction of particles entering the gel phase.

The effect of \bar{D}_g on the DGT response is straightforward. The ratio between the flux for the colloidal complex system and that for the blank (metal only) is

$$\frac{J}{J_{\text{blank}}} = \frac{\bar{D}_g c_{Tgl} / \delta_g}{D_M c_T / \delta_g} = \frac{c_{Tgl}}{c_T} \frac{(1 + \varepsilon K'_g)}{(1 + K'_g)} \quad (8)$$

in which c_{Tgl} refers to complexes that are *both penetrating and labile*. Note that K'_g may differ from K' due to differences between c_{Mpg} and c_{Mp} as resulting from incomplete penetration by the particulate species. The similar ratio for DET signals is simply

$$c_g^* / c_{g,\text{blank}}^* = c_{Tg} / c_T \quad (9)$$

in which c_{Tg} signifies the total concentration of all penetrating species. The crucial feature is that the DET response is not linked to ε . A numerical example to illustrate the important difference between the DGT and DET response is given in Table 1.

	DGT	DET
signal ratio	J/J_{blank}	$c_{\text{Tg}}/c_{\text{T}}$
$\varepsilon = 10^{-2}$	0.02	1
$\varepsilon = 10^{-1}$	0.11	1

Table 1. Example of DGT versus DET signal ratios for different sizes of the colloidal complexes. The computation is based on eqs 8 and 9, assuming all species are fully penetrating and labile. $K' = 100$, $\varepsilon = D_{\text{Mp}} / D_{\text{M}}$, $c_{\text{M}}^* = 0.01 \times c_{\text{T}}^*$ and $c_{\text{Mp}}^* = 0.99 \times c_{\text{T}}^*$.

In brief, full penetration would yield a DET ratio of unity and a DGT ratio of 0.02 (labile) or 0.01 (inert). The DGT signal for colloidal complex systems is often assumed to represent the free metal only (11). For a complex with $K' = 10^2$ and $\varepsilon = 10^{-2}$ this results in an overestimation of the free metal ion concentration by a factor of 2, while with increasing K' the error increases. In the typical nanoparticle range with ε on the order of 10^{-2} to 10^{-1} , the error is already large for $K' = 10^2$. All of this illustrates that DET reflects the mere permeation whereas DGT is the dynamic mobility/labability discriminator for the permeating species.

Colloidal complexes are DGT labile if they dissociate sufficiently fast on the timescale of their diffusion through the gel layer (7). The lability criterion derives from the ratio between the kinetically controlled metal ion flux and the diffusion-controlled flux of the colloidal complex, and is usually given in a form such as

$$A\delta_{\text{g}} \gg 1 \quad (10)$$

with for $\delta_{\text{g}} \gg \delta_{\text{sln}}$, the lability parameter A defined by

$$A = \frac{k_{\text{d}}^{1/2}(1 + \varepsilon K')^{1/2}}{D_{\text{p}}^{1/2} \varepsilon K'} \quad (11)$$

where k_{d} is the dissociation rate constant of the colloidal complex. For $\varepsilon K' \gg 1$, i.e. complexes so strong that in spite of their reduced mobility they dominate over the free metal term in the overall diffusion flux, the expression for A reduces to the well-known limit (6)

$$A = (k_{\text{d}}/D_{\text{p}} \varepsilon K')^{1/2} \quad (12)$$

2.3. Experimental

2.3.1. Set-up of the DGT and DET

The DGT technique was performed using regular Solution Deployment Units from DGT Research in Lancaster. The diffusive gels and Chelex ion-exchange resin gels were homemade and stored until use, according to the protocol provided by DGT Research. The gels used for DET were harvested from the same stock of diffusive gels. The gels used were cast at 0.50 mm thickness for the open pore gel (resulting in 0.84 mm final thickness) and 0.25 mm for the restricted gel (0.42 mm final thickness). The water volume fraction (α) is 0.95 and 0.84 for the open pore and restricted gel types respectively (11). Prior to deployment, the gels were equilibrated in a solution with the same ionic strength as in the experiments for at least 48 hours. DET experiments were carried out with the gel floating freely in solution, which means that the effective δ_g is only half the total layer thickness. Dispersions were stirred at all times.

2.3.2. Latex particles

Two different samples of monodisperse, carboxylated latex nanospheres with radii 40.5 nm and 129 nm (see AFM picture in Figure S1 in the supporting information) were obtained from Ikerlat Polymers (Spain). These nanoparticles were cleaned by the manufacturer, who provided a conductimetric and potentiometric characterization of each sample. The particles have also been previously studied (25, 26) providing us with extensive information on particle diffusion coefficients and ion binding affinities for various metals, including Pb(II), and various pH and ionic strength conditions; see Table S1 in the supporting information. This table includes the expected values for crucial parameters like ε and $\varepsilon K'$, as calculated from the species diffusion coefficients and stability constant for the given metal/particle complex system.

Apart from their being well characterized and excellently monodisperse, the main interest in using these particles as model ligands is their $\varepsilon K'$ value under our experimental conditions. For the pH values and particle concentrations used in this study, $\varepsilon K'$ values are on the order of 1, while the K' values are of order 10^2 . Any colloidal complex penetration into the gel layer should then be clearly reflected by the difference between the DGT and DET signal ratios as outlined above.

2.3.3. Other experimental equipment

All glassware used was acid-washed in several steps using HNO_3 (ultrapure, Fluka) and ultrapure water. Elution of metal from the gels was performed with HNO_3 directly in polystyrene tubes fit for ICP-MS trace metal analysis. Data here was analyzed by ICP-MS

(Perkin Elmer; Elan 6000). Graphs were plotted and fitted using GraphPad Prism 4 for Windows.

2.3.4. Experimental setup

Fresh dispersions were prepared separately for each latex particle size. The dispersion volume was at least 150 ml, sufficient to prevent bulk depletion of lead. The total Pb(II) concentration was 3.31×10^{-6} M, prepared from Pb^{2+} ion-standard, Metrohm. Latex particle dispersions were 0.10 % (mass) with roughly equal amounts of surface ligand groups for the two types of particles. MES buffer (from MES sodium salt, Sigma), and HNO_3 were used to fix the pH at 6.2, and 0.01 M NaNO_3 (pro analysis salt, Merck) was added to prevent Donnan effects. The temperature is 22 °C and volumes are kept airtight to prevent evaporation over time. DGT and DET were performed simultaneously. The DGT deployment units were extracted after various time intervals. DET gels were extracted after at least 20 hours.

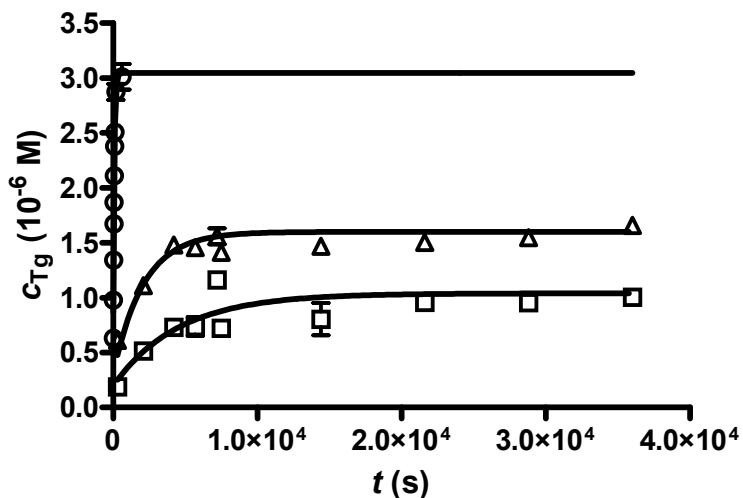
2.4. Results and discussion

2.4.1. DET

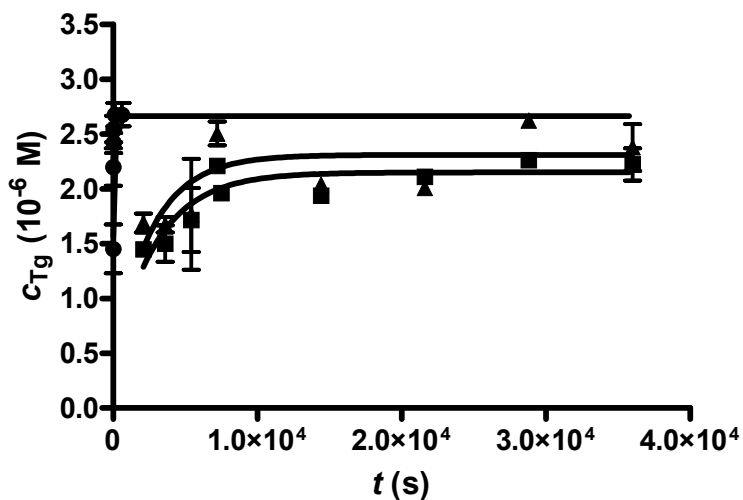
Table 2a collects the DET results. The values measured for c_{Tg}^* are much larger than c_{M}^* which shows that latex particles in the size range up to 129 nm radius enter the diffusion gel. The error for all experimental data was within 10%, except for that of c_{Tg}^* for the 129 nm particle in the open pore gel which was larger (1.04 ± 0.26). The directly measured c_{Tg}^* is at least 50 times larger than the free metal concentration indicating that particle-bound metal penetrates the gel. The total metal concentration in the gel is on the order of 10^{-6} M; metal-only experiments show that specific binding of the metal by the gel matrix (19) is insignificant at our concentration levels. Control experiments for concentration levels of 10 nM total metal also yielded no detectable enhancement outside the experimental error. Penetration takes place to an appreciable extent, that is up to 30 to 50% of the total particle concentration in the sample for the open pore gel. Remarkably, the extent of penetration is even higher for the restricted gel (65 to 70%). The presence of particle-bound metal in the gel is even more clearly shown by the ratio between the concentrations of particle-bound metal in gel and in solution, $c_{\text{Mpg}}/c_{\text{Mp}}$, see Table 2a. The value of c_{Mpg} is simply calculated from c_{Tg}^* assuming that unbound metal is free to enter the gel, i.e. $c_{\text{Mg}}/c_{\text{M}} = \alpha$, the water volume fraction. Particle penetration is then reflected

by $c_{\text{Mpg}}/c_{\text{Mp}}$ which can take any value between 0 and α . For all gels and particles investigated, $c_{\text{Mpg}}/c_{\text{Mp}}$ is found to be in the range between 0.3 and 0.7. None of the dispersions investigated reaches the limit of full penetration (α) which suggests that part of the gel volume is inaccessible to the particles in the investigated size range 40-129 nm.

Using the value of c_{Mpg} , \bar{D}_g was calculated according to eq 6, assuming that ε in the gel is equal to ε in the sample medium. The effective value of \bar{D}_g is also reflected in the growth of the mean metal concentration in the gel with DET exposure time. Experimental data are shown in Figure 1. The top curves (\circ, \bullet) show the time dependence of $c_{\text{Tg}}(t)$ for the blank metal-only case. After appropriate volume correction, the equilibrium concentration (c_{Tg}^*) is the same as the metal concentration in solution. The faster equilibration for the restricted gel is simply due to its smaller thickness, in accordance with eq 2. In the presence of particles, the characteristic timescales of the DET equilibration transients are appreciably longer than for the metal-only case. This reflects the lowering of \bar{D}_g and independently confirms that particle-bound metal enters the gel phase. The rate of diffusion in the gel phase can be estimated using the exponential equilibration function given by eq 2. Values obtained for the corresponding rate constant ratio k/k_{blank} (with $k = \pi^2 \bar{D}_g / 4\delta_g^2$), are listed in Table S2 in the supporting information.



(a)



(b)

Figure 1. Temporal DET equilibration profiles for Pb(II) in the case of free Pb only (\circ , \bullet) and in the presence of 40.5 nm particles (Δ , \blacktriangle) and 129 nm particles (\square , \blacksquare). Open and closed symbols are used for the cases of open pore gels (figure 1a) and restricted gels (figure 1b) respectively. Full curves are fits with eq 2; see Table S2 in the supporting information for parameter values. An appropriately scaled figure showing only free Pb data is available in the supporting information.

For open pore gels, the 129 nm particle dispersion clearly has a lower c_{Tg}^* than the 40.5 nm dispersion. This is not surprising since one would expect the abundance of pores, large enough to house the 129 nm particles, to be lower than those for smaller particles, which in turn would be lower than the volume available for free metal ions. Consequently, \overline{D}_g for the particle dispersions are larger than \overline{D} in bulk solution. For the same reason \overline{D}_g for the 129 nm particle case would be expected to be higher than that for the 40.5 nm particles provided their K' is the same.

For the restricted gel, the free metal ion diffusion coefficient is lower than for the open pore gel (13). Particle diffusion coefficients are also found to be lower than in the open pore gel, but the eventual particle concentration is higher. The equilibrium concentration of total metal, c_{Tg}^* , in the restricted gel is almost independent of particle size. There is a slight trend that concentrations are lower for larger particles, but the differences are not as pronounced as for the open pore gels. This implies that for restricted gels, the fraction of gel space which is available to particles, is practically equally accessible to all particles in the size range studied. The casting of restricted gels differs from that of the open pore ones in that more crosslinker is added in order to lower the average pore size. The resulting water content is only 84% compared to the 95% of the open pore gel. Formation of the restricted gel is faster, and this might give rise to stronger structural inhomogeneity with a wider pore size distribution. The experimental data seem to suggest that this results in even more large pores in the restricted gel than in the open pore gel. Such a pore distribution might explain why c_{Tg}^* for a colloidal complex is ultimately higher. The increased inhomogeneity in restricted gels also seems to be reflected by a larger variation in equilibrium metal concentration between different batches of gels (see also Figure 1).

Recent research on agarose gels (14, 15, 27) indicates that pores near the gel surface are actually smaller than the average pore in the gel, while the bulk of the gel can also contain void volumes, i.e. relatively large pockets of solution in the bulk gel body. Although agarose and polyacrylamide gels are structurally different, it could be that such porous inhomogeneity is a more general feature of gels, with significant consequences for their permeability.

gel type	a (10^{-9} m)	c_{Tg}^* (10^{-6} M)	c_{Mpg}/c_{Mp}	\overline{D}_g (10^{-12} m ² s ⁻¹)
Open Pore	40.5	1.60 ± 0.13	0.48	20.5
	129	1.04 ± 0.26	0.31	8.55
Restricted	40.5	2.31 ± 0.28	0.70	11.2
	129	2.15 ± 0.13	0.47	4.37

Table 2a. DET results for total Pb^{2+} concentration c_{Tg}^* in the 40.5 and 129 nm latex dispersions and ensuing ratio between particle-bound metal in gel and that in solution (c_{Mpg}/c_{Mp}). The mean diffusion coefficient in gel (\overline{D}_g) is computed using eq 6. It is assumed here that $\varepsilon_g = \varepsilon$.

	actual	Open Pore	Restricted
a (10^{-9} m)	c_M/c_T	J/J_{blank}	J/J_{blank}
40.5	$9.5 \cdot 10^{-3}$	$1.59 \cdot 10^{-2}$	$1.48 \cdot 10^{-2}$
129	$2.9 \cdot 10^{-3}$	$3.01 \cdot 10^{-3}$	$3.88 \cdot 10^{-3}$

Table 2b. DGT flux ratios of Pb^{2+} in the 40.5 and 129 nm latex dispersions as compared to the theoretical signal ratio (c_M/c_T).

2.4.2. DGT

It has been shown before that, under the present experimental conditions, the Pb(II)/carboxylated latex complex is partly labile on the effective timescale of stripping chronopotentiometry (SCP) at a conventional mercury drop electrode (28). Since the characteristic timescale of DGT, δ_g^2/D , is by several orders of magnitude larger than that of SCP (6), the Pb(II)/latex complex is expected to be completely DGT-labile. For our experimental results, the DGT response for the colloidal complex system is enhanced compared to the signal for the free Pb^{2+} only, while the extent of enhancement is in proportion to the concentration of particle-bound lead in the gel, as measured by DET. The general picture of the DGT signal ratio for the Pb^{2+} /latex dispersion is shown in Table 2b. In DGT, the ratio between sample signal and blank signal ranges around 10^{-3} and 10^{-2} , whereas the DET ratio varies from 0.3 to 0.7. For the 129 nm particle in the open pore gel

the difference between the DGT ratio and the actual c_M/c_T is the smallest and prone to large error. As explained in the theory above, this is due to the small value of D_p/D_M and the ensuing comparability of the terms for free metal and bound metal in \bar{D} , i.e. $\varepsilon K'$ is of order unity. It is precisely for this feature that the present combination of DGT with DET is particularly profitable: DET determines the total concentration of free metal and gel-permeating metal/particle complex. Using that, the DGT flux ratio, and eq 8 yields the K' in the gel phase, i.e. K'_g . The relation between this K'_g and K' in the original sample dispersion is simply given by the excluded volume factors of the particle species (α_p) and the free metal species (α_M), provided there are no Donnan effects (18, 19) or specific binding. The resulting concentrations in the sample dispersion have been back-calculated (Table S3 in the supporting information). When comparing this DET/DGT-derived speciation to the parameters obtained by stripping chronopotentiometry (22, 23) (Table S1), the resulting values for c_{Mp} and K' are very consistent indeed.

It may be concluded that DET experiments with Pb (II)/latex complexes clearly show that latex particles of 40 and 129 nm enter the gel to a significant extent. The size range of particles that penetrate the gel is well above what has been assumed thus far. In DGT, contributions to the flux by gel-permeating colloidal metal complexes are masked by their inherently lower diffusion rates. Data interpretation on the basis of presumed impermeability leads to overestimation of the free metal concentration in the sample. It is recommended that DGT speciation analysis of samples possibly containing colloidal species, be supported by parallel DET experiments which yield the sum of free and particle-bound metal in the gel. Since DGT measures the mobility-weighted form of this sum, the combination of DET with DGT allows for computation of both concentrations, provided the mobility ratio is known.

Supporting Information Available

Speciation characteristics of Pb^{2+} /latex dispersions (Table S1), equilibration rate constants and mean diffusion coefficients in gel (Table S2), experimentally calculated values of K' (Table S3), AFM data of nanospheres (Figure S1) and the temporal DET equilibration profiles (Figure S2).

2.5. Supporting information

a (10^{-9} m)	40.5	129
D_{Mp} (10^{-12} m ² s ⁻¹)	5.83 ± 0.22	1.74 ± 0.02
c_{LT} (10^{-5} mol COOH l ⁻¹)	5.2	5.9
$\log K'$	2.0	2.5
c_M (10^{-8} M)	3.2	0.96
c_{Mp} (10^{-6} M)	3.28	3.30
\bar{D} (10^{-12} m ² s ⁻¹)	13.0	3.89
ε (10^{-3})	8.08	2.38
$\varepsilon K'$	0.84	0.82

Table S1. Speciation characteristics (a , D_{Mp} , c_{LT} , K') and calculated values for Pb^{2+} /latex dispersions used. Particle concentration 0.10 % (mass). pH = 6.18. Ionic strength = 0.01 M. $[Pb(II)]_T = 3.31 \cdot 10^{-6}$ M. Diffusion coefficient of free lead ion = $7.40 \cdot 10^{-10}$ m²s⁻¹, also equal to the diffusion coefficient of free lead in the open pore gel at 22 °C (11).

gel type	a (10^{-9} m)	k/k_{blank}	\bar{D}_g (10^{-12} m ² s ⁻¹)
Open Pore	40.0	$38 \cdot 10^{-3}$	34
	129	$18 \cdot 10^{-3}$	16
Restricted	40.0	$5.5 \cdot 10^{-3}$	6.7
	129	$4.9 \cdot 10^{-3}$	6.0

Table S2. Equilibration rate constants k and corresponding mean diffusion coefficient in the gel (\bar{D}_g) as measured by time-dependent DET and application of eq 2.

gel type	a (10^{-9} m)	c_M (10^{-8} M)	c_{Mp} (10^{-6} M)	$\log K'$
Open Pore	40.0	3.8	3.27	1.9
	129	0.7	3.30	2.7
Restricted	40.0	2.3	3.29	2.2
	129	0.6	3.30	2.8

Table S3. Resulting values of K' (c_{Mp}/c_M) in the bulk sample from the combined DGT and DET results (Table 3) on the basis of $K_g = K$ in the sample, taking into account the appropriate excluded-volume corrections (see text).

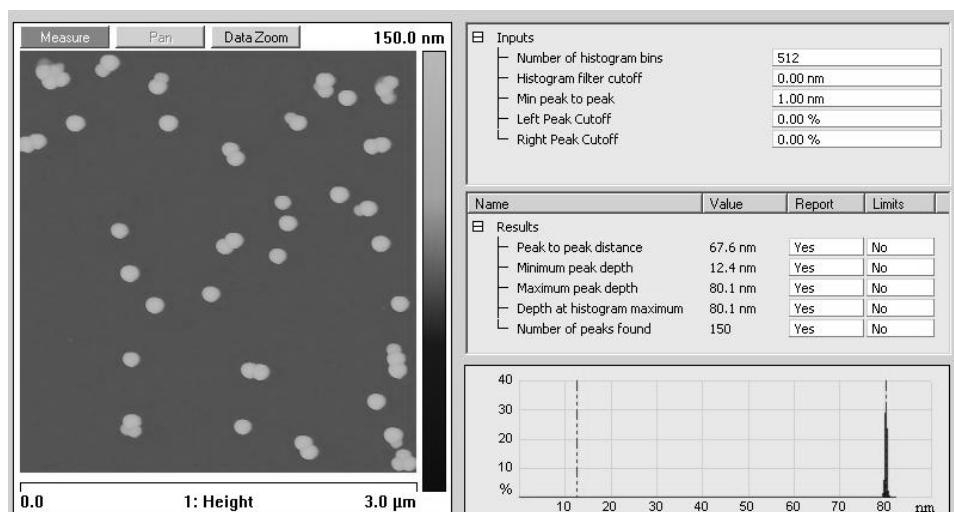


Figure S1. Atomic Force Microscopy (AFM) image of the 40.5 nm radius carboxylated latex nanospheres and the corresponding AFM particle size distribution analysis.

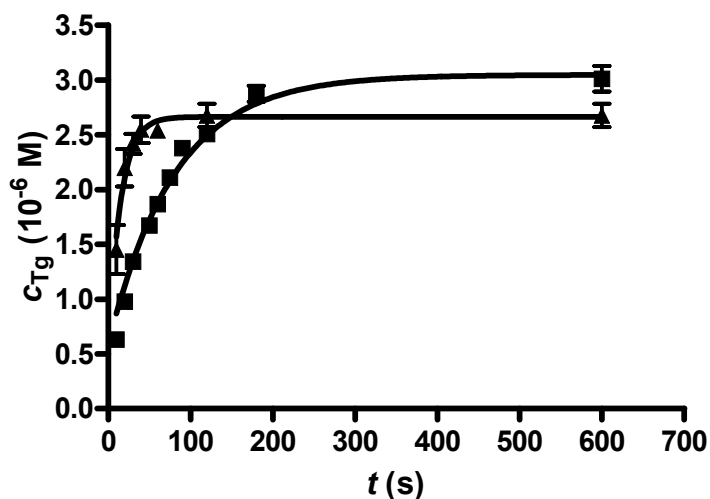


Figure S2. Temporal DET equilibration profiles for Pb(II). Total lead concentration in the absence of ligand for open pore gel (■) and restricted gel (▲). Full curves are fits with eq 2; see Table S2 for parameter values.

2.6. References

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Chapter 3

Donnan effects in metal speciation analysis by DET/DGT

Lee P. Yezek, Pascal L.R. van der Veeken, Herman P. van Leeuwen

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Abstract

Crosslinked polyacrylamide gels have found wide application in analytical techniques such as Diffusional Equilibration in Thin Film (DET) and Diffusive Gradient in Thin Film (DGT). In these applications, the assumption is made that the gel matrix is effectively uncharged and chemically inert with respect to the species of interest. Recent data has shown significant non-ideal behavior at low ionic strengths, which strongly suggests a finite structural charge within the gel matrix. The present investigation explores the possible ramifications of fixed charged sites within the gel on metal speciation analysis by DET and DGT. The results indicate that structural charge within the gel layer will give rise to different DET equilibrium concentrations than in the sample, due to Donnan partitioning. The steady-state diffusion of ions through a diffusive gel (DGT) is also influenced by the gel charge, but the net effect will depend on the details of the speciation. The results indicate that for submillimolar ionic strength solutions the quantitative interpretation of DGT measurements runs into problems which are largely overcome by combination with DET.

3.1. Introduction

High water content gels of crosslinked polymer networks have found wide application in analytical techniques (1-6). In metal speciation analysis, for example, both the Diffusional Equilibration in Thin Film (DET) (7) and Diffusive Gradient in Thin Film (DGT) (1, 2) techniques make use of a gel phase made of crosslinked polyacrylamide. DET is based on establishing species partition equilibrium between the gel and a metal solution. The gel sensor is allowed to equilibrate with the solution of interest and the equilibrium concentration of penetrating metal species in the gel is determined analytically. DET is thus sensitive to the concentration of free metal ions and bound metal in the form of complexes small enough to enter the gel sensor. DGT, on the other hand, relies on the on-going diffusion of species through a gel matrix on their way to a collector. By quantifying the amount of metal bound by the collector in a known deployment time, the steady-state flux and solution concentration of available metal in the form of free metal ions and labile complexes can be computed.

DGT has proved to be a very successful means for in-situ measurement of the concentration of free metal and labile complexes in natural waters (8, 9). However, problematic results have been reported for deployments in low ionic strength natural waters (10, 11). Alfaro-De la Torre et al. first reported “enhanced diffusion coefficients” of metal ions in low ionic strength waters compared to a control sample in 0.01 M sodium nitrate (12). The effect was observed for a variety of metals and freshwaters, and the specific significance of low ionic strength was confirmed in synthetic freshwater solutions (13, 14). Clearly, the increased fluxes reported in these studies reflect an enhanced concentration of diffusing species within the diffusive gel, rather than an actual increase in the mobility of the ions themselves. Recently, the enhanced concentrations of positive metal species within the gel have been shown to result from the presence of fixed negatively charged sites within the polymer matrix (15). The purpose of the present investigation is to explore the possible ramifications of structural fixed charged groups within the gel on the analytical performance of DGT and DET in dynamic speciation analysis of natural freshwaters.

3.2. Theory

3.2.1. Gel Structure

The gels used in DET and DGT are normally made of crosslinked polyacrylamide. The layers used in speciation analysis have a typical thickness δ_g on the order of 1 mm (1, 2), hence for all ionic strengths δ_g is much larger than the Debye length, κ^{-1} . As a

consequence, the electrical properties of the gel can be considered in terms of a bulk equilibrium Donnan potential difference. Thus, if the gel possesses fixed charged groups, of valence z_g and concentration N , bound within the structure of the gel itself, a Donnan potential difference, Ψ_D , will develop between the bulk gel environment and the bulk electrolyte solution. An expression for the equilibrium Donnan potential between the two phases emerges from the condition of overall electroneutrality in the bulk gel (16):

$$\Psi_D = \frac{RT}{zF} \operatorname{arcsinh}\left(\frac{z_g N}{2zc}\right) \quad (1)$$

where $z_+ = z_- = z$ is the valence of the (symmetrical) supporting electrolyte of bulk concentration c , R is the gas constant, T the absolute temperature, and F the Faraday constant. Eq 1 holds for a single symmetrical electrolyte and is thus limited to cases where the concentration of the metal ion of interest is low relative to the total electrolyte so as not to influence the Donnan potential. If this condition is not met, the Donnan potential expression will still emerge from the electroneutrality condition, but the contribution of metal ions and their counterions must be explicitly taken into account (see e.g. (17)).

3.2.2. Ideal Donnan Equilibrium

A finite Donnan potential has consequences for the partitioning of all ions between the gel phase and the electrolyte solution. The equilibrium Donnan partitioning coefficient, Π_D , is simply defined by the pertaining Boltzmann factor (15):

$$\Pi_D = \frac{c_g}{c} = \exp(-zF\Psi_D/RT) \quad (2)$$

where c_g represents the concentration within the gel of an ion of charge z and bulk solution concentration c^* . For the typical case of an anionic gel, the equilibrium concentration of all cationic species will be enhanced within the gel relative to the bulk solution, whereas all negative species will have diminished concentrations within the gel phase. The enhancement or reduction of concentration within the gel is a function of the Donnan potential and the valence of the ion in question, independent of the chemical identity of the species. It does not reflect specific binding to the gel of any kind whatsoever, but is the mere electrostatic consequence of the potential difference between the bulk gel environment and the bulk solution, due to the presence of fixed charged sites within the polymer network.

3.2.3. Transport: steady-state diffusion through charged gels

A schematic diagram of a standard DGT device is given in Figure 1. The device consists of a strongly binding resin, which acts as a metal accumulator, covered by a high water content (84 – 95% (18)) diffusive gel made of polyacrylamide.

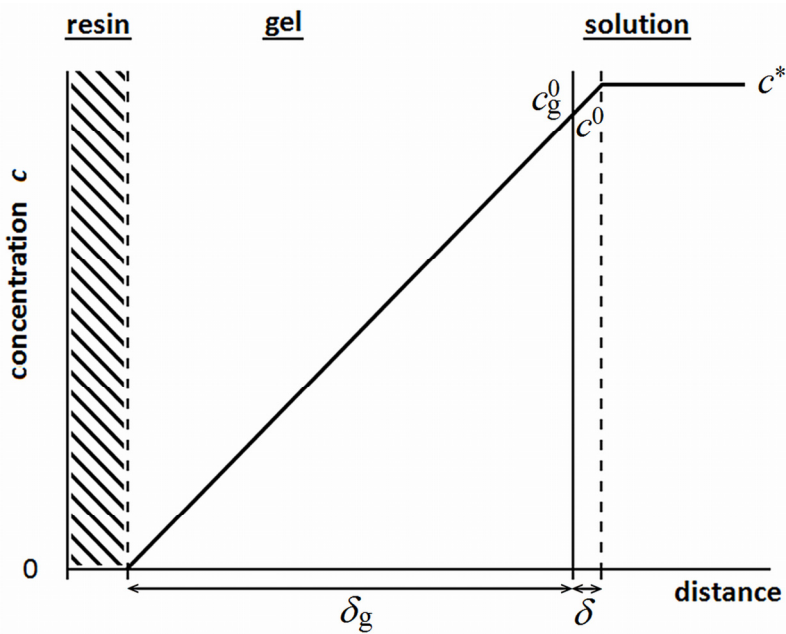


Figure 1. Schematic diagram of a standard DGT device: Resin gel layer, diffusive gel layer (thickness δ_g), the diffusion layer in solution (δ) and bulk solution concentration c^* together with the resulting concentration profile.

Figure 1 also depicts the steady-state concentration gradient which develops as a result of diffusion of metal into the gel phase, driven by the sink function of the backing layer of resin. For typical deployment conditions in DGT, the thickness of the diffusion layer in solution, δ , is on the order of 100 μm , i.e., about 10% of the thickness of the diffusive gel, which is usually on the order of 1 mm. The characteristic time constant governing the process of setting up a steady-state concentration gradient, τ , is on the order of δ_g^2 / D_g , where δ_g is the thickness of the gel layer and D_g the diffusion coefficient in the gel phase. For $\delta_g \approx 1 \text{ mm}$ and D_g in the range of 10^{-10} to $10^{-9} \text{ m}^2\text{s}^{-1}$, τ is on the order of an hour (19).

In the steady-state the flux of metal ions within the diffusion layer in the sample solution, J_s , is simply given by:

$$J_s = \frac{D_s(c^* - c^0)}{\delta} \quad (3)$$

where c^0 represents the metal ion concentration at the solution side of the gel/solution interface. Thus $(c^* - c^0)/\delta$ is the concentration gradient in the solution diffusion layer and D_s represents the diffusion coefficient of the species of interest in solution. If more than one type of species is present, for example free metal and labile complexes, an average diffusion coefficient is used to describe all diffusing metal species (18).

$$\bar{D} = \frac{D_M c_M^* + \sum_i D_{ML_i} c_{ML_i}^*}{c_M^* + \sum_i c_{ML_i}^*} \quad (4)$$

In the case of a significant difference between D_M and D_{ML} , the value of the average diffusion coefficient is sensitive to the distribution of metal ions between the free and bound forms.

In the analysis of DGT data, the resin is assumed to be a perfect sink for metal ions (1), provided the extent of binding to the resin remains far below saturation levels. Under such conditions, the steady-state flux of metal ions through the diffusive gel to the binding resin, J_g , is given by (15):

$$J_g = \frac{D_g c_g^0}{\delta_g} = \frac{D_g \Pi_D c^0}{\delta_g} \quad (5)$$

where c_g^0 is the metal concentration at the gel side of the gel/solution interface. In eq 5, c_g^0/δ_g represents the steady-state concentration gradient which develops within the diffusive gel, with the Donnan partitioning coefficient, Π_D , represented in c_g^0 . It is tacitly assumed that Donnan equilibrium is indeed achieved at the gel/solution interface, which is reasonable since generally there is no interfacial ion transfer resistance. Since the polymer network of the gel reduces the free volume available for diffusion, D_g may be expected to be somewhat lower than D_s (21, 22) For the gels used in commercially available DGT, D_g/D_s values for simple ions range from 0.8 to close to 1.0 (18), depending on the nature and concentration of cross-linker in the polymer network. This is mainly linked to excluded

volume as related to the water volume fraction (α) of these gels. Sets of equations that take into account this excluded volume effect are provided in the supporting information.

Under steady-state conditions, the fluxes through the two phases are equal, that is, $J_s = J_g = J$. After substitution between eqs 3 and 5, and elimination of c^0 , the steady-state flux from solution to the resin layer is obtained as (15):

$$J = \frac{D_g c^*}{\frac{\delta_g}{\Pi_D} + \delta \frac{D_g}{D_s}} = \frac{c^*}{\frac{1}{\Pi_D} \frac{\delta_g}{D_g} + \frac{\delta}{D_s}} \quad (6)$$

If the DGT gel is essentially uncharged, or the ionic strength high enough to suppress electrostatic effects, eq 6 reduces to a simplified, Donnan-free expression (1) for the steady-state flux:

$$J = \frac{c^*}{\frac{\delta_g}{D_g} + \frac{\delta}{D_s}} \quad (7)$$

For complex sample solutions, this equation differentiates to

$$J = \frac{c_t^*}{\frac{\delta_g}{\bar{D}_g} + \frac{\bar{\delta}}{\bar{D}_s}} \quad (8)$$

where c_t^* is the total concentration of species that are entering the gel and are DGT-labile, \bar{D}_g and \bar{D}_s are the average D inside the gel phase and in solution respectively, and $\bar{\delta}$ is the thickness of the diffusion layer in solution (which is a function of \bar{D}_s (23)). Note that this equation ignores the transport contribution of complexes in solution that are labile but not able to enter the gel phase (20).

For systems that essentially follow eq 6, it is convenient to define a diffusive transport coefficient, y , as the ratio of the two terms in the denominator (15):

$$y = \frac{D_s \delta_g}{\Pi_D D_g \delta} \quad (9)$$

Then for $y \gg 1$, diffusion within the gel is limiting and J approaches J_g (eq 5), whereas for $y \ll 1$ diffusion in solution is limiting and J approaches J_s (eq 3). Normally, if Π_D equals unity, then $y > 1$.

From the definition of flux and substitution into eq 6, the increase of the mass, m , of metal accumulated in the resin gel of cross-sectional area, A , with time, t , is given by:

$$\frac{\Delta m}{\Delta t} = \frac{c^* D_g A}{\frac{\delta_g}{\Pi_D} + \delta \frac{D_g}{D_s}} \quad (10)$$

As the total metal bound to the collecting resin can be determined analytically, rearranging eq 10 allows the concentration of available metal in the bulk solution, c^* , to be calculated from $\Delta m/\Delta t$ at steady-state conditions. An important point to note is that, as the value of Π_D increases, the length of time required to set up the steady-state concentration gradient in the diffusive gel will increase. If the diffusive gel is charged, Donnan partitioning acts to develop an *enhanced* concentration *gradient* within the diffusive gel and an ensuing greater relative impact of diffusion in solution. The rate at which the concentration gradient develops in the gel is dependent on the rate of diffusion from solution, hence the time necessary to set up the steady-state concentration gradient will increase with the magnitude of Π_D . It approaches a linear dependence on the limit of high Π_D with control by diffusion in solution, the latter reaching steady-state faster than diffusion in the gel. It is the filling of the gel layer up to the steady-state concentration profile that requires significant time. Thus for significantly charged gels at low ionic strengths, τ can increase to several hours. It is also of importance that the concentration of the diffusing metal ions of interest be negligible relative to the concentration of the supporting electrolyte. If this is not the case, the diffusing ions themselves can significantly impact on the magnitude of the Donnan potential in the diffusive gel (17). The result would be a Donnan potential gradient in the diffusive gel, which would greatly complicate the interpretation of experimental data.

3.3. Discussion

Eq 6 demonstrates the importance of Donnan partitioning on the steady-state flux of ions in a DGT device. Enhanced rates of metal uptake at the collector have been reported in polyacrylamide gels at low ionic strengths (10, 11). Yezek and van Leeuwen (15) used electrokinetics and conductivity measurements to estimate the magnitude of the Donnan potential difference in methylene-bis-acrylamide cross-linked polyacrylamide gels and reported Donnan potentials on the order of -10 mV for NaCl concentrations on the order of 1 mM. Such Donnan potentials are of a magnitude comparable to those responsible for the apparent “diffusional enhancements” reported by Sangi et al. (11) in the mM concentration regime. Therefore in the paragraphs that follow, the impact on speciation analysis by DET

and DGT of a -10 mV Donnan potential difference between the gel phase and the bulk electrolyte solution will be discussed. Considering the low charge nature of the gels normally used in DET and DGT, the discussion below can be taken as a worst case scenario when considering average global freshwater conditions. Still, in exceptional cases of low ionic strength freshwaters the effects may be considerably more pronounced. A few specific cases of significant analytical interest are discussed below.

3.3.1. Free metal ions only

If the test solution contains metal in the form of the free metal ion only, **DET** will provide a solution concentration of a given ion which will deviate from the real value by a factor of Π_D (or $\alpha\Pi_D$ if excluded volume effects are taken into account), as defined in eq 2. As a result, divalent free metal ion concentrations would be overestimated by a factor of approximately 2.1 if the Donnan potential of the gel is -10 mV. In order to observe such an enhancement, it is imperative that the deployment time is long enough to reach equilibrium (note that this is longer than in the Donnan-free case).

The impact of Donnan effects on the steady-state diffusion of metal ions in **DGT** was the topic of a recent investigation (15). Provided the Donnan potential is non-zero, two limiting situations can be identified for eq 6. Figure 2a illustrates the concentration gradient which develops across the diffusive gel for $y \gg 1$. The concentration of metal ions at the gel side of the gel/solution interface, c_g^0 , is given by $c_g^0 \approx \Pi_D c^0$ and $c^0 = c^*$. The flux through the diffusive gel is thus enhanced relative to what would be expected for an uncharged gel by a factor approximately equal to Π_D .

If $y \ll 1$, the thickness of the diffusion boundary layer, δ , plays the crucial role in determining the magnitude of the steady-state flux of ions in the diffusive gel. Specifically, under steady-state conditions, the flux of ions in the diffusive gel can never exceed the maximum possible flux in the solution diffusion layer, which has a limiting value of $J_s = D_s c^* / \delta$, regardless of how large Π_D may become. Figure 2b very clearly makes the point, although this case corresponds to a deliberately extremely large Π_D normally not encountered in nature. The slope of the line representing the concentration gradient in the diffusive gel must be equal to the slope of the line depicting the concentration gradient in the diffusion layer. As the slope of the line describing the flux in the diffusion layer approaches the limiting value of $D_s c^* / \delta$, the flux in the diffusive gel approaches the same limit. It is the precise but very small value of c^0 that varies with Π_D . For $\Pi_D > 1$, c^0 is given by c_g^0 / Π_D , which approaches zero for large Donnan potentials. If the Donnan potential is at some intermediate value of e.g. -10 mV, neither limiting case applies, and eq

6 must be used to analyze the experimental data. For a diffusive gel layer thickness of 0.9 mm, a solution diffusion layer thickness of 100 μm , and a Π_D of 2.1 for a divalent ion (corresponding to $\Psi_D = -10$ mV), eq 6 provides a flux enhanced by a factor of 1.9 relative to an uncharged gel ($\Pi_D = 1$). This analysis assumes that the diffusion coefficient of the metal ions in the gel is not affected by the magnitude of the Donnan potential. If the solution diffusion layer is thicker, for example 200 μm , the flux for the charged gel is enhanced by factor of 1.8 relative to the uncharged case, while a thickness of 50 μm would show an enhancement by a factor of 2.0. Thus, the magnitude of the possible error depends on the hydrodynamics in the deployment site as well as on the ionic strength (and hence the magnitude of the Donnan potential).

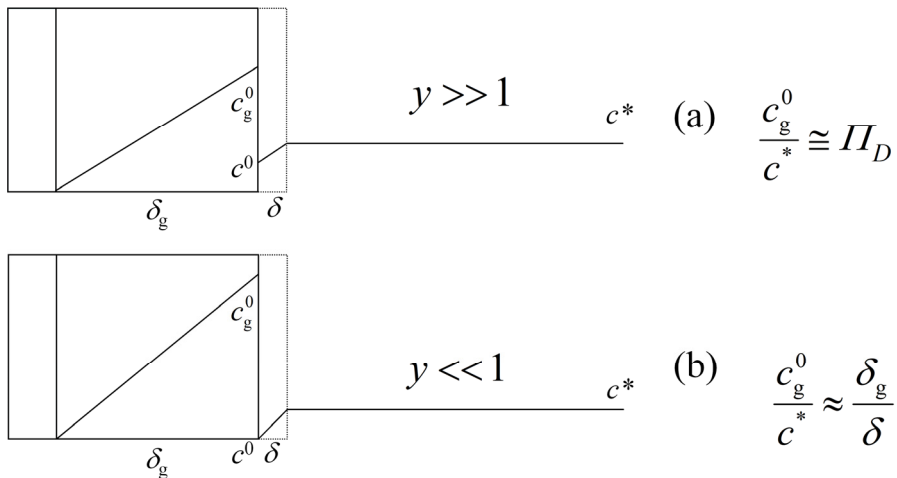


Figure 2. Sketch of the steady-state concentration gradient across the diffusion layer in solution (δ) and the diffusive gel for the two limiting cases of y , as defined by eq 9. Interfacial ion concentrations are the concentration at the solution side (c^0) and the gel side (c_g^0) of the gel/solution interface. The case of $y \gg 1$ corresponds to a modest Π_D , whereas the $y \ll 1$ case corresponds to an extremely large Π_D which is normally not encountered in natural systems. For $y \ll 1$, $D_g \approx D_s$ since $J_g = J_s$.

3.3.2. Free metal ions and large labile complexes

If the test solution is assumed to consist of only free metal ions plus large complexes and ligands, which are assumed to be unable to enter the gel phase, the potential impacts on **DET** are the same as discussed in the previous section. If these large complexes are labile, the situation for **DGT** measurements is not identical to what was discussed above. The

effective diffusion coefficient in solution will then be an average over the labile and free species and the thickness of the solution diffusion layer is derived from that (23). As the complexes are generally larger than the free metal ions, the average diffusion coefficient of available metal will be smaller than that of the free metal ions in solution. Since in this case there is coupled diffusion of free and bound metal in solution, but only the smaller species penetrate the gel phase, diffusion in solution will become more important than in the case of free metal alone. It also demonstrates that in such a case the use of a single diffusion coefficient for both the solution and the gel introduces a rather significant approximation.

3.3.3. Free metal ions and penetrating labile complexes

If the test solution is assumed to consist of free metal ions, M^{2+} , and sufficiently small, labile complexes with a ligand, L , of charge z_L , the equilibrium between bound and free forms within the gel is described by:



$$K = \frac{[ML^{2+z_L}]_g}{[M^{2+}]_g [L^{z_L}]_g}$$

where the brackets indicate concentrations and the subscript g denotes the gel phase. The penetrating labile complexes are also subjected to an excluded volume effect, which generally differs from that for the small ions (for details see the supporting information). The concentrations of each form within the gel phase are given by:

$$\begin{aligned} [M^{2+}]_g &= [M^{2+}]_s \Pi_{D,M} \\ [L^{z_L}]_g &= [L^{z_L}]_s \Pi_{D,L} \\ [ML^{2+z_L}]_g &= [ML^{2+z_L}]_s \Pi_{D,ML} \end{aligned} \quad (12)$$

where the subscripts M, L, and ML refer to the metal ion, ligand, and complex, respectively, and s refers to the electrolyte solution. The concentrations of all ionic species within the gel are subject to Donnan partitioning according to eq 2. In a negatively charged gel the concentrations of positive metal ions and complexes will be enhanced, while negatively charged ligands will be to some extent excluded from the gel. The net result is that both the speciation and the total concentration of metal within the gel phase will differ relative to the external solution. The equilibrium ratio of free to bound metal within the gel phase is given by:

$$\frac{[M^{2+}]_g}{[ML^{2+z_L}]_g} = \frac{1}{K[L^{z_L}]_g} \quad (13)$$

Apart from uncharged ligands such as NH_3 , the ratio of free to bound metal within the gel phase is a function of the Donnan potential, and hence the ionic strength of the external solution. In the most usual case of a negatively charged gel and negatively charged ligand, the ratio will shift in favor of the free metal at low ionic strengths, meaning $\Pi_{D,M} > 1$ and $\Pi_{D,L} < 1$, with $\Pi_{D,ML}$ in between.

The rate of steady-state diffusion of labile metal species in DGT depends on the concentration gradient which develops within the diffusive gel. In the limiting case where $y \gg 1$, the flux is limited by diffusion in the gel phase, and the Donnan partitioning will control the magnitude of the concentration gradient, exactly as depicted in Figure 2a. The concentration of each species at the gel side of the gel/solution interface can be approximated by the expressions in eq 12. The resulting total flux will be the sum of the fluxes of the labile species in the gel phase. The average diffusion coefficient in the gel phase is an average over the bound and free forms which will also depend on the magnitude of the Donnan potential, see eq 13. As a result, both the total metal concentration and the average diffusion coefficient will change as a function of ionic strength.

In the case of $y \ll 1$, the flux of ions is controlled by diffusion in solution, as discussed above and depicted in Figure 2b. Because the complexes considered are labile, only the coupled diffusion of free metal ions and complexes of ligands is relevant. Since their charges are usually different, the speciation in the gel will differ from that in the bulk solution. As a result, the average diffusion coefficient in the gel will assume a value different from that in solution, and the steady-state concentrations of free and bound metal in the gel will be dictated by the equilibria described in eqs 11-13, and the pertaining transport equation 8.

A Donnan potential of -10 mV does not correspond to either of the limiting situations described above. The magnitude of the various effects on the total bound metal within a given deployment time, t , which is the directly measurable quantity in DGT, will depend on the speciation in solution and the charges of the various complexes. Table 1 provides an illustrative example for 3 possible situations with free metal and one complex at $\Psi_D = -10$ mV. For each case, the total concentration of metal is 1.0, distributed in three different ways between the free and bound states. The first two examples assume a complex of charge +1, the last one a complex of charge -1. The metal and complex concentrations as well as the ratio of the free to bound metal both outside and inside the gel (assuming Donnan equilibrium holds) are given, as the ligand is negatively charged and the ratio of free to bound metal in the gel phase will be significantly different from that in solution. In

addition, the total concentration of available metal changes as well. It can be either increased, as is the case in the two examples with complexes of +1 charge, or reduced, as in the last example. As long as the ligand maintains large excess over metal in the gel phase, the changes in average diffusion coefficient are relatively modest. Hence it is the changes in concentration within the gel due to Donnan partitioning which are primarily responsible for the deviations of the flux from the values expected assuming an uncharged gel. Depending on the speciation of metal in solution, the Donnan effects yield a net increase or, if the dominant species are negatively charged, a net decrease in the DGT flux. If neither charge is dominant, as in the third example in Table 1, the net flux can be changed in either direction, depending on the charge and relative concentration of the complexes.

Species	$[M]_s$	$[ML]_s$	$[M]_g$	$[ML]_g$	$\frac{[M]_s}{[ML]_s}$	$\frac{[M]_g}{[ML]_g}$	$[M]_g + [ML]_g$
M ²⁺ ML ⁺	0.8	0.2	1.743	0.295	4	5.9	2.038
M ²⁺ ML ⁺	0.2	0.8	0.435	1.18	0.25	0.369	1.616
M ²⁺ ML ⁻	0.2	0.8	0.435	0.542	0.25	0.804	0.977

Table 1. Theoretical equilibrium concentrations in DET of free metal and complexes in solution and in the gel phase for $\Psi_D = -10$ mV.

3.3.4. Implications for experimental setup

If low ionic strength waters are of interest, it is desirable to calibrate the DGT sensors with synthetic test solutions of ionic strength matching that of the sample. This will provide a means of estimating the magnitude of the Donnan potential present in the diffusive gel. However, the interpretation of DGT results at very low ionic strengths is still problematic, as the magnitude of the required Donnan correction factor will depend on the specifics of the speciation, as indicated in Table 1. DGT in principle provides only a measure of metal available for binding to the chelex resin in the device, rather than a detailed speciation.

It may be concluded that in DET, being an equilibrium technique, Donnan partitioning would provide total metal concentrations in the gel phase which are biased by the partitioning term for each type of charged species. Complexes too large to enter the gel

phase make no contribution: recent data show that the permeability of larger species is differentiated and significant up to the 100 nm regime (24). In DGT, a steady-state flux technique, the picture is substantially more complicated. The Donnan partitioning affects not only the total metal concentration in the gel phase, but also the relative concentrations of all charged species, see eqs 11-13. Hence, the metal speciation within the gel phase depends on the magnitude of the Donnan potential, which is a function of the overall ionic strength of the medium. Consequently, the relationship between the amount of metal measured by DGT and the corresponding metal species distribution in the bulk sample solution is rather involved, and is ionic strength dependent. It is thus very difficult to quantitatively interpret DGT results for a completely unknown water sample at low ionic strengths.

Supporting Information Available

Equations that take into account excluded volume as related to the water volume fraction of the gel.

3.4. Supporting Information

Rigorous application of hydrogels in metal speciation analysis calls for consideration of the exclusion of species by the volume of the gel material itself. The volume fraction available for metal species within the gel, is related to those areas in the gel not occupied by gel material but water, the water volume fraction, α . Species partitioning is then reflected by the ratio between the species concentrations inside and outside the gel, c_g^0/c^0 , which can take any value between 0 and α (or $\alpha\Pi_D$ if Donnan effects are relevant). The zero limit is the case of no penetration at all, whereas α corresponds to the limit of ideal partition. Here we outline the modification of the various equations in the main text, for proper accounting of the α term.

In the application of the definition of flux, as in eq 10, α is included as follows (see also the derivation of eq S-5 below):

$$\frac{\Delta m}{\Delta t} = \frac{\alpha c^* D_g A}{\frac{\delta_g}{\Pi_D} + \alpha \delta \frac{D_g}{D_s}} \quad (\text{S-1})$$

From the definition of y , the ratio of the two elements in the denominator of eq S-1, an adapted y can be defined as

$$y = \frac{D_s \delta_g}{\alpha \Pi_D D_g \delta} \quad (\text{S-2})$$

For a sample solution with free metal and penetrating labile complex species we have an effective $\bar{\alpha}$ defined in parallel with \bar{D} eq 4:

$$\bar{\alpha} = \frac{\alpha_M c_M^* + \sum_i \alpha_{ML_i} c_{ML_i}^*}{c_M^* + \sum_i c_{ML_i}^*} \quad (\text{S-3})$$

This $\bar{\alpha}$ is also applied when looking at the concentrations of each form within the gel phase, as in eq 12, since each species may have a different water volume fraction available.

$$\begin{aligned} [M^{2+}]_g &= [M^{2+}]_s \Pi_{D,M} \alpha_M \\ [L^{z_L}]_g &= [L^{z_L}]_s \Pi_{D,L} \alpha_L \\ [ML^{2+z_L}]_g &= [ML^{2+z_L}]_s \Pi_{D,ML} \alpha_{ML} \end{aligned} \quad (\text{S-4})$$

For macromolecular ligands, α_{ML} and α_L are approximately the same.

For the steady-state flux of metal ions in DGT as given in eq 5, α is incorporated in a manner equivalent to that of Π_D :

$$J_g = \frac{D_g c_g^0}{\delta_g} = \frac{\alpha D_g \Pi_D c^0}{\delta_g} \quad (\text{S-5})$$

It follows that eq 6 modifies to

$$J = \frac{\alpha D_g c^*}{\frac{\delta_g}{\Pi_D} + \alpha \delta \frac{D_g}{D_s}} \quad (\text{S-6})$$

which becomes evident from the two limits of eq S-5: $D_s c^* / \delta$ for control by diffusion in solution, and $\alpha \Pi_D D_g c^* / \delta_g$ for control by diffusion in the gel layer. Correspondingly, the Donnan-free expression of eq 7 changes into

$$J = \frac{\alpha c^*}{\frac{\delta_g}{D_g} + \frac{\alpha \delta}{D_s}} \quad (\text{S-7})$$

As already indicated, α has a complex version, $\bar{\alpha}$. So the complex version of eq S-7 develops to

$$J = \frac{\bar{\alpha} c_t^*}{\frac{\delta_g}{D_g} + \frac{\bar{\alpha} \delta}{D_s}} \quad (\text{S-8})$$

which applies to the total concentration of species that are entering the gel *and* are DGT-labile. It still ignores the transport contribution of complexes in solution that are labile but not able to enter the gel phase.

3.5. References

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Chapter 4

Accumulation of Humic Acid in DET/DGT gels

Pascal L.R. van der Veeken, P. Chakraborty, Herman P. van Leeuwen

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Abstract

Gel layer based sensors are increasingly employed for dynamic trace metal speciation analysis in aquatic and soil media. In DGT (Diffusive Gradient in Thin film), polyacrylamide hydrogels are commonly used for the diffusive gel layer. Whilst some effects of humic and fulvic acids on the DGT detection of metal species have been observed, the gel permeation properties of the actual humic and fulvic acid compounds themselves have not been analyzed thus far. Here we show with DET (Diffusive Equilibrium in Thin film) that these natural complexing agents do enter the gel layer, and that humic acids even appear to accumulate in the gel, with enrichment factors typically on the order of ten. The results have consequences for the interpretation of DGT-data on metal fluxes from aquatic media containing humics and fulvics.

4.1. Introduction

Over the last decades, research into aquatic metal ion species has made significant progress, for a large part on the level of dynamic speciation (1-5). Several techniques have been proposed for analyzing the properties of a variety of metal species and timescales. They include a range of sensors based on diffusive gel layers (6, 7). Today, Diffusive Gradients in Thin film (DGT) and, to a lesser extent, Diffusive Equilibration in Thin film (DET) (8-10) are known techniques that are based on diffusion of metal ion species into a gel layer.

In DET, the gel layer is kept in contact with the sample solution until partition equilibrium with the sample is achieved. The final total metal content of the gel phase reflects the physical ability of complex species to enter the gel phase, so size is the controlling parameter in this method. DGT, on the other hand, effectively employs the gel layer as a diffusion layer between the sample solution and a strongly complexing resin layer, which acts as a perfect metal ion sink. In the usual setting of DGT, this results, after a relatively short transient stage, in a steady-state flux of the sufficiently labile species and a corresponding process of metal accumulation in the resin layer. After a certain deployment time, the amounts of different metals in the resin are eluted and quantified with a suitable method such as ICP-MS. The amount found for a certain metal represents the integral of the diffusion flux of this metal over the deployment time. As with DET, the possible participation by larger complexes depends on the effective pore sizes of the diffusion gel layer. However, in contrast with DET, the DGT signal also depends on the mobilities of the participating species inside the gel layer.

The impact of complexing agents like humic substances on dynamic metal ion speciation in aquatic media is crucial (4). Such substances are highly complex and diverse, and are found in a large variety of natural settings, from soils and sediments to marine waters and freshwaters. Fulvic acids are smaller in size than humic acids, while the latter have lower functional group density (mainly carboxylic and phenolic) (11, 12). Many of them, especially the humic acids, also have amphiphilic properties. This is mainly caused by the abundance of functional groups at the outer part of the HA as compared to the more hydrophobic inner structure. The environmental abundance and ligand traits of humic and fulvic acids imply they are also of major importance for the bioavailability of heavy metals (13).

Research into the effects of humics on metal ion transport in DGT is facing uncertainties concerning the gel/sample partition behavior of the HA and FA compounds themselves. Due to their complexing properties, their presence or absence in the gel itself could have a significant impact on metal transport in the gel. If these substances would

even accumulate in the gel layer, their impact would be even more drastic, as the speciation inside the gel would be changed as compared to that in the sample medium.

Two different types of polyacrylamide gel are commonly used in DET and DGT. They are generally referred to as the “open pore gel” and the “restricted gel”. This paper will focus on the permeation behavior of HA and FA with the open pore gel type, in the absence of metal ions. Although pertaining research is ongoing (14-16), specific structural properties of the gels have not been fully determined yet. This is not only the case for polyacrylamide-based gels; it is only recently that the first exploratory fluorescence correlation spectroscopy (FCS) studies were carried out on fluorophores in agarose gels (17-19).

In the past, the diffusive gel pores of the polyacrylamide-based gels have been assumed not to exceed 10 nm (20). Recent work on colloidal complexes (14) has shown however, that the pore size of the gels is substantially larger than that. The new estimates on the pore size exceed the typical sizes of HA and FA species. As a consequence, these compounds may be expected to be able to enter the gel phase. Since FA and HA carry charge, other phenomena such as Donnan effects (15, 21, 22) may also influence the partition of HA and FA between sample solution and gel at lower ionic strength. All together, this will generate a concerted modification of the speciation and dynamics of metal ions under DGT measurement conditions. We shall tackle the problem in two steps: first we investigate the simple partition characteristics of HA and FA for different concentrations and ionic strengths, and second we shall analyze the impact of FA and HA on the DGT and DET signals of metal ions. The present paper deals with the first step.

4.2. Theory

4.2.1. Fluxes in DET/DGT

DET (20, 23) is performed by bringing a gel layer in contact with the sample solution and waiting long enough for solute partition equilibrium to fully establish itself between gel and sample. The resulting total solute concentration in the gel (c_g^*) is then measured. The evaluation is based on the usual conditions that (i) the initial concentration in the gel phase is zero, (ii) transfer of penetrating species across the water/gel interface does not involve any kinetic limitation, so that the equilibration process is generally diffusion-controlled, (iii) the steady-state diffusion layer in solution is thin compared to the gel layer thickness and the solution volume is much larger than the gel layer volume, so that the solution concentration (c_w^*) remains essentially constant, (iv) Donnan effects, if any, are taken into account (15, 21, 22), and (v) interactions with the gel matrix are insignificant.

Since partition equilibrium with the sample is established, c_g^* becomes essentially equal to the sample concentration of the pertaining species, provided they can freely enter the gel, and the excluded volume effect in the gel phase is properly accounted for.

As outlined before (14), the time needed to establish partition equilibrium is derived from the elementary diffusion time constant τ , defined by the distance δ_g and the diffusion coefficient D_g (24):

$$\tau = \frac{\delta_g^2}{D_g} \quad (1)$$

Although DET is usually applied to metals, it may also yield information on permeating humic and fulvic acids, as long as a suitable method for quantifying their concentrations in the gel phase is available.

In DGT, the thickness of the gel layer is usually chosen sufficiently large (order 10^{-3} m) to render the diffusion in solution (layer thickness on the order of 10^{-4} m (4)) largely insignificant. According to Fick's first law, the flux (J) of a solute through the gel layer then depends on its diffusion coefficient in the gel (D_g), the diffusion distance (δ_g) and the difference between the concentration in solution (c_w^*) and that at the boundary between the gel and resin layer (c'). Under conditions as listed for DET, the flux J then is:

$$J = \frac{D_g (c_w^* - c')}{\delta_g} \quad (2)$$

Since the initial concentration in the gel layer is assumed to be zero, a certain time is needed for the system to establish steady state transport. The magnitude of this time derives from the diffusion-controlled τ defined in eq 1. See refs (14, 25, 26) for details. In the case of heterogeneous compounds like humic/fulvic acids, differences in the sizes of different species of HA and FA entering the gel will impact on the equilibration rate (DET) and the steady-state achievement time and magnitude of the eventual flux (DGT).

4.2.2. Donnan effects

The gel layers used in DET and DGT have a typical thickness δ_g on the order of 1 mm (8), hence for all ionic strengths δ_g is much larger than the Debye length, κ^{-1} . Thus, if the gel possesses fixed charged groups, a Donnan potential difference will develop between the bulk gel environment and the bulk electrolyte solution (22, 27). Such a Donnan potential has consequences for the partitioning of all charged species between the gel phase and the solution. Therefore, the presence of humic or fulvic acid in the gel would render

any humic or fulvic acid species susceptible to this electrostatic term in its partitioning. Moreover, if the humic or fulvic acid concentration differs from the one outside the gel, it would also alter the diffusion of any metal with which it forms complex species. These effects will be significant especially at the lower ionic strengths typical to freshwater systems.

4.3. Experimental

4.3.1. Humic and fulvic acids

Humic acid used was a forest soil humic acid (FSHA) (28-30), which has previously been used in gel based sensor experiments. It was originally extracted from forest floor material taken from the Tongbersven forest (Oisterwijk, The Netherlands). Suwannee River Fulvic Acid (SRFA) standard was obtained from IHSS (Cat. No. 1S101F), and originally extracted from Suwannee River (Georgia, U.S.). These compounds have also been previously studied (28-32) providing us with information on elemental content and concentrations of acidic functional groups, as collected in Table 1.

	Molecular Weight (kDa)	Elementary Composition (%(w/w))			Acidic Functional Groups (mol kg ⁻¹ C)	
		C	H	O	Carboxyl	Phenolic
FSHA	22	52.9	5.4	39.3	4.35	7.41
SRFA	2.31	52.44	4.31	42.20	11.44	2.91

Table 1. Elemental content and acidic functional groups for purified humic acid from the Tongbersven (FSHA) and Suwannee River fulvic acid standard (SRFA).

4.3.2. Set-up of the DET

The diffusive gels were homemade and stored until use, according to the protocol provided by DGT Research in Lancaster. The diffusive gels used were cast at 0.50 mm thickness (resulting in 0.84 mm effective total gel layer thickness in aqueous medium). The water volume fraction (α) for this gel type (open pore) is 0.95 (20), and this value has been applied in correcting c_g^* for all data points. Below 10 mM ionic strength the gel is negatively charged at the pH of the experiments (6.1) (22, 33). DET experiments are carried

out with the gel floating freely in the sample medium, which implies that the effective δ_g is only half the total gel layer thickness. Dispersions are gently stirred at all times.

4.3.3. Experimental setup

All glassware was given a multi-step acid-wash prior to use to ensure no metal contaminations using HNO_3 (ultrapure, Fluka) and ultra pure water. Fresh dispersions were prepared for each experiment. The dispersion volume was 1000 ml, with 0.001-1 M added NaNO_3 (pro analysis salt, Merck). HA and FA concentrations are in the range of 0.3-10 mg L^{-1} and 2.6-50 mg L^{-1} respectively. The ranges of these concentrations are of the same order as found in common natural systems, while being sufficient for detection by UV/Vis spectroscopy. MES buffer (from MES sodium salt, Sigma, and HNO_3) was used to keep the pH constant at 6.1. During gel layer deployment the temperature is kept constant at 22 °C and volumes are kept airtight to prevent evaporation over time. Preliminary measurements showed that the deployment time of two weeks used in all experiments was sufficient to determine equilibrium concentrations.

The polyacrylamide gel discs were prepared for UV/Vis measurement straight from dispersion, with at least 4 replicates per measurement (standard deviation is used for any error bars in the presented data). After extraction, a quick square cutout is made of 1 cm^2 , to ensure a good fit into the standard sized UV/Vis cuvette. The cutout is mounted vertically, such that the light beam traverses the gel material perpendicular to the square surface. The wavelength range of the UV/Vis measurements was 244-500 nm. No difference in spectrum characteristics of FA/HA between water and gel phase was observed. The largest UV background signal in this range is caused by NO_3^- , with a relative minimum compared to FA/HA at a wavelength of 280 nm. Thus this wavelength was selected for the measurements in this work. In cases of significant accumulation, the observed change in intensity was clearly visible to the bare eye. All spectroscopic measurements were performed on a Hitachi U-2010 UV/Visible spectrophotometer.

4.4. Results and discussion

4.4.1. SRFA

Figure 1 collects the DET results for SRFA equilibrium concentrations in the gel for different bulk concentrations of SRFA. Under the conditions mentioned in the theoretical section, including the correction of c_g^* for excluded volume, a c_g^*/c_w^* ratio of 1 implies full partition, but no accumulation. Most of the data points indeed show full partition of the FA between gel and aqueous medium. Some cases even show a slight accumulation (with

c_g^*/c_w^* between 1 and 1.5). For the lower ionic strength (2 mM) and the lowest FA sample concentrations the partition coefficient remains below unity ($c_g^*/c_w^* < 1$). In the case of 1 M ionic strength, no accumulation effects were observed; within the experimental error the c_g^*/c_w^* ratio is unity for all FA concentrations (data not shown).

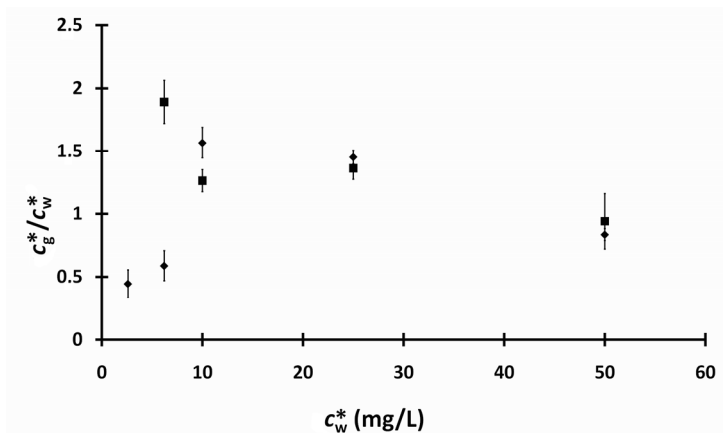


Figure 1. Normalized SRFA accumulation in DET at ionic strengths of 11 mM (■) and 2 mM (◆) as a function of bulk SRFA concentration.

The FA is known to be easily soluble in water, and is therefore not expected to have a large hydrophobic affinity for the gel material. The DET gel used here is also known to generate an appreciable Donnan potential on the order of -10 mV at 2 mM ionic strength, but it is practically negligible above an ionic strength of 10 mM (22, 33). The SRFA is expected to be negatively charged at pH 6.1, which would explain the lower fulvic acid concentrations in the gel in the presence of a negative Donnan potential, although this effect is not apparent at higher concentrations. Indeed, for sufficiently large ionic strengths that ensure suppression of Donnan effects (11 mM series), the fulvic acid seems to accumulate somewhat (see the discussion on humics below).

4.4.2. FSHA

Figures 2a and 2b collect the FSHA accumulation data for 11 mM and 2 mM ionic strength, respectively. Both graphs not only show that HA enters the gel, but also that a substantial accumulation of HA takes place ($c_g^*/c_w^* \gg 1$). There is a clearly linear relationship between the HA concentration in the gel and that in the sample medium, with a large difference between the accumulation coefficients for the different ionic strengths. As with fulvic acid, no accumulation effects could be detected for very high ionic strength samples (1 M), which all show c_g^*/c_w^* ratios around unity for all HA concentrations (data not shown). In passing, we mention that gels with HA pre-accumulated at low ionic strength, release HA upon re-immersion in high ionic strength (1 M) solution, demonstrating the reversibility of the accumulation.

From the sole standpoint of electrostatic Donnan effects on partitioning, the higher accumulation factor of HA for the lower ionic strength is at first sight counterintuitive. However, it has been shown that humic acids are to some extent amphiphilic and therefore capable of forming micelle-like structures (11, 12, 34). Since these humic micelles consist of different unidentical HA entities, these micelles are usually referred to as ‘pseudomicelles’. Due to reduced electrostatic screening of the charged groups, aggregates of this type are less favorable at lower ionic strength. The precise formation properties of humic pseudomicelles are very sensitive to the physicochemical conditions in the medium, as well as to the type of humic acid. The observed accumulation of FSHA in the polyacrylamide gel suggests a substantial hydrophobic affinity of the FSHA for the gel matrix. Then, if the ionic strength is so low that the charged parts prevent the hydrophobic regions of the humic acid to aggregate, these would be free to interact with the polymer backbone of the gel. As a result, the affinity of FSHA for the gel phase would increase with decreasing ionic strength, as observed in the experimental data.

Figure 3 shows the course of the accumulation process with time, for a bulk FSHA concentration of 2 mg L^{-1} . Note that the HA concentration inside the gel is non-uniform as it’s approaching its eventual equilibrium value c_g^* ; therefore the plot shows the average HA concentration over the whole gel layer (\bar{c}_g).

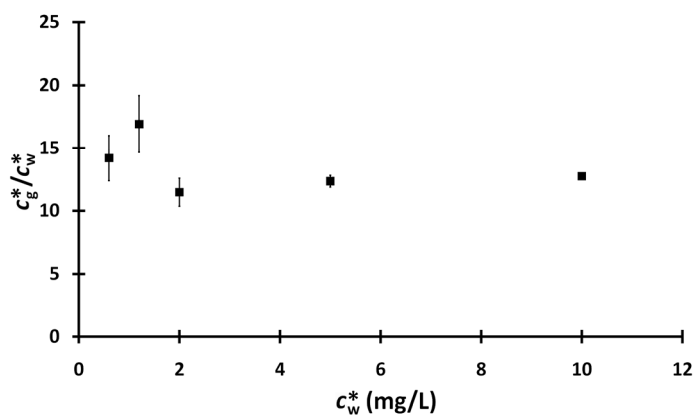


Figure 2a. Normalized FSHA accumulation in DET at an ionic strength of 11 mM as a function of bulk FSHA concentration.

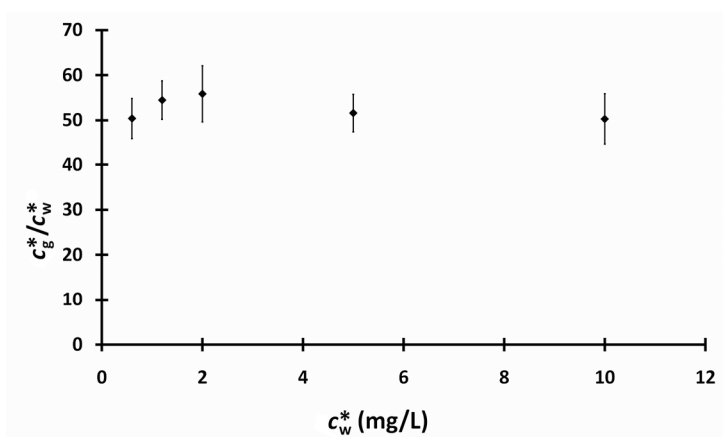


Figure 2b. Normalized FSHA accumulation in DET at an ionic strength of 2 mM as a function of bulk FSHA concentration.

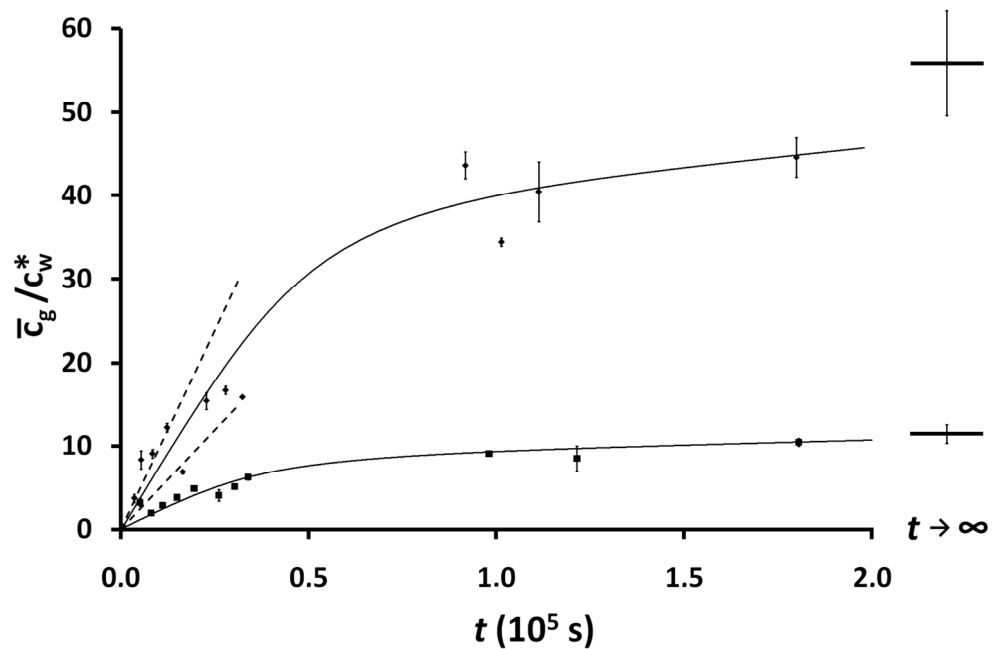


Figure 3. Normalized FSHA accumulation in DET at ionic strengths of 11 mM (■) and 2 mM (◆) at a bulk concentration of 2 mg L^{-1} as a function of time. Curves are added to guide the eye. Dashed lines indicate the initial linear shape of the accumulation curve. Equilibrium levels of \bar{c}_g/c_w^* displayed on the right-hand side by a horizontal bar for $t \rightarrow \infty$.

Since the accumulation is quite strong, it may be speculated that the initial stage of the diffusion of the humic acid into the gel phase is largely limited by its diffusion in solution (note that steady-state transport in solution is established much faster than in the gel layer). Thus we calculated the limiting steady-state flux in the medium, assuming a constant δ_w of 50 and 100 μm , together with a D_w of 2×10^{-11} and $8 \times 10^{-11} \text{ m}^2\text{s}^{-1}$, for 11 mM and 2 mM ionic strength respectively. The corresponding initial slopes are included in the curves of fig 3 and convincingly coincide with experimentally observed slopes. The lower slope for the higher ionic strength confirms the aggregation of HA to ‘pseudomicelles’ with lower D_w .

In contrast to the early stage of equilibration, where the process is limited by diffusion in solution, the timescale for accumulation τ_{acc} for the 2 mM ionic strength case appears longer than that of the 11 mM case. This would seem to point to a stronger interaction of the FSHA with the polymer backbone, and a correspondingly larger accumulation demand. The resulting total accumulation, represented by c_g^*/c_w^* indeed indicates a substantially higher affinity of the smaller FSHA units to the gel, as discussed above.

Finally, it is expected that the timescale of this accumulation profile in itself will already have a significant effect on any DGT that is applied to such a system, as it will take a large amount of time to resolve the transient stage. In DGT this gel has an effective δ_g of 0.84 mm, suggesting it would take around 10^5 to 10^6 seconds (several days) to establish a real steady-state flux. At lower ionic strength, a large effect from accumulated HA on the charge properties inside the gel is also expected. As follows from Table 1 and Figure 2b, $[\text{COO}^-]$ inside the gel will be on the order of 1 mM which will significantly modify the Donnan potential.

It may be concluded that DET experiments with FSHA and SRFA on DET/DGT gels clearly show that these substances do not only enter the gel phase, but in the case of FSHA also accumulate by a factor of tens. The accumulation is especially strong at relatively low ionic strength, implying a very serious accumulation in freshwater conditions. Media with an ionic strength similar to marine conditions show no accumulation. It will be very useful to study the accumulation properties for different types of humic acids, and to see how they compare to this soil type HA for various ionic strengths, from freshwaters to marine levels.

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Chapter 5

DGT/DET gel partition features of humic acid/metal species

Pascal L.R. van der Veeken, Herman P. van Leeuwen

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Abstract

Gel layer based sensors are increasingly employed for dynamic trace metal speciation analysis in aquatic and soil media, in which humic and fulvic acid species are generally known to be relevant. In DGT (Diffusive Gradient in Thin Film), polyacrylamide hydrogels are commonly used for the diffusive gel layer. Various effects of the presence of humic species on the amount of metal detected by DGT have been observed, but the role of the different metal/humic species is still unknown. Recently it was shown that in the absence of metal, humic acid accumulates significantly in the polyacrylamide hydrogel. Here we analyze the extent of this accumulation in the presence of cadmium under various conditions of ionic strength and total humic and fulvic acid concentrations. At millimolar ionic strength level, DET data show significant accumulation of cadmium(II) in the gel phase, on top of some Donnan enrichment. The results are essential for the interpretation of DGT/DET data on metals in the presence of humics, especially in freshwaters.

5.1. Introduction

Dynamic metal speciation analysis has become an important element of research into aquatic metal ion species (1-5). Different experimental techniques have been proposed for metal speciation analysis over a range of timescales, including a suite of sensors based on diffusive gel layers (6, 7). Diffusive Gradients in Thin film (DGT) and Diffusive Equilibration in Thin film (DET) (8-10) are techniques based on passive permeation of metal ion species into a gel layer with well-defined dimensions.

DET analyses the eventual partition equilibrium between the gel layer and the sample. The size of complex species is decisive for their entering the gel phase; it therefore is the primary controlling parameter. In contrast, DGT employs the gel layer as a controlled diffusion layer backed by a metal ion sink, usually in the form of a strongly complexing resin layer. In the standard DGT deployment method, sufficiently labile species contribute to the flux of metal to the resin layer. Within the steady-state regime, the increase in the amount of metal accumulated in the resin layer with deployment time then represents the overall diffusion flux of all contributing metal species. As with DET, the physical ability of species to enter the gel layer governs their contribution to the accumulated amount. By its nature, the DGT signal also depends on the mobilities and labilities of the participating species inside the gel layer. Thus for complicated samples, the different features of DGT and DET may be expected to combine fruitfully in dynamic metal speciation analysis.

Humic substances are common complexing agents in aquatic media, and have a very significant impact on metal ion speciation in a large variety of natural settings (4). Fulvic acids (FA) and the larger humic acids (HA) are highly complex and diverse, with varying amounts of carboxylic and phenolic functional groups (11, 12). The distributed nature of the thermodynamic and kinetic features of fulvic and humic metal complexes strongly impacts on the bioavailability of metals (13).

Two different types of polyacrylamide gel, commonly used in DET and DGT are usually denoted as the “open pore gel” and the “restricted gel”. The present work focuses on the permeation/partition behavior of HA and FA for the open pore gel type, in the presence of metal ions in the sample. The detailed structural properties of the gel have not been fully determined yet, although research is ongoing (14-16). This is also true for other gels used in dynamic speciation; recently, the first exploratory fluorescence correlation spectroscopy (FCS) studies were carried out on agarose gels (17-19). Studies on colloidal complexes (14) have shown that the pore size of the polyacrylamide-based gels is substantially larger than the previously assumed order of 10 nm (20). The pore size certainly exceeds the typical sizes of HA and FA, and these species have indeed been observed to enter the gel phase (21). In the case of humic acid, there is even significant

accumulation, to an extent that strongly depends on the ionic strength of the system (21). For HA it is known that the functional metal ion binding groups are largely located at the outside of the molecular entity. Many humic substances, especially the humic acids, therefore have amphiphillic properties and form micellar secondary structures. The effect of ionic strength on the accumulation of HA in the gel is presumably related to this pseudomicellar behavior (12, 21). Due to their complexing properties, the presence or absence of FA/HA in the gel itself potentially has a significant impact on metal ion transport in the gel. Furthermore, in the case of accumulation in the gel layer, their impact could be even more drastic, as the speciation inside the gel would be changed as compared to that in the sample medium. Since FA and HA carry charge, other phenomena such as Donnan effects (15, 22, 23) may also influence the partition of these compounds between sample solution and gel at lower ionic strength. All together, this will generate a concerted modification of the speciation and dynamics of metal ions under DGT measurement conditions. With the partition characteristics of FA and HA for different concentrations and ionic strengths at hand (21), the present paper will focus on the simultaneous gel/sample partitioning of fulvics/humics and their metal complexes, using Cd^{2+} as a representative complexing ion.

5.2. Theory

5.2.1. General conditions

DET (9, 20, 24) is performed by bringing a gel layer in contact with the sample solution and waiting long enough for solute partition equilibrium to fully establish itself between gel and sample. The resulting total solute concentration in the gel (c_g^*) is then measured. The evaluation is based on the usual conditions that (i) the initial concentration in the gel phase is zero, (ii) transfer of permeating species across the water/gel interface does not involve any kinetic limitation, so that the equilibration process is generally diffusion-controlled, (iii) the steady-state diffusion layer in solution is thin compared to the gel layer thickness and the solution volume is much larger than the gel layer volume, so that the solution concentration (c_w^*) remains essentially constant, (iv) Donnan effects, if any, are taken into account (19, 25, 26), and (v) interactions with the gel matrix are insignificant. Since in DET partition equilibrium with the sample is fully established, c_g^* becomes essentially equal to the sample concentration of the pertaining species, provided they can freely enter the gel, and the excluded volume effect in the gel phase is properly accounted for. Although DET is usually applied to metals, it may also yield information on

permeating humic and fulvic acids, as long as a suitable method for quantifying their concentrations in the gel phase is available.

5.2.2. Donnan effects

The gel layers used in DET and DGT have a typical thickness δ_g on the order of 1 mm (8). This implies that for all ionic strengths δ_g is much larger than the characteristic electrostatic screening distance, that is the Debye length κ^{-1} . Since the gel generally possesses a small amount of fixed charged groups, a Donnan potential difference will develop between the bulk gel environment and the bulk electrolyte solution in the lower ionic strength regime (26, 27). Such a Donnan potential has consequences for the partitioning of all charged species between the gel phase and the solution. A Donnan-modified humic or fulvic acid concentration inside the gel would also alter the diffusion of any metal with which it forms complex species. The situation is exacerbated by the fact that humic entities may behave as Donnan particles themselves and exhibit electrostatically modified metal binding and diffusion properties. All these effects will be significant, especially at the lower ionic strengths typical to freshwater systems.

5.2.3. DET equilibrium

Previous research (21) has shown the extent to which the Forest Soil HA (FSHA) and Suwannee River FA (SRFA) accumulate in the polyacrylamide hydrogel at different ionic strengths. The data for FSHA suggest a substantial hydrophobic affinity of the FSHA for the gel matrix. The underlying reason for this accumulation is the amphiphilic nature of humic acids, which make them capable of forming micelle-like structures (11, 12, 28). Since the humic micelles consist of a number of unidentical HA entities, they are usually referred to as ‘pseudomicelles’. The precise formation properties of humic pseudomicelles are very sensitive to the physicochemical conditions in the medium, as well as to the type of humic acid. Then, if the ionic strength is so low that intermolecular electrostatic repulsion prevents aggregation, the hydrophobic regions would have more freedom to associate with the polymer backbone of the gel, giving rise to an increase in affinity of FSHA with decreasing ionic strength.

In the presence of metal complexes of these humics, e.g. Cd/HA, the different species and their basic association/dissociation reactions are given schematically in figure 1. Since the binding characteristics of cadmium with FSHA have been reported (29), all concentrations of species outside the gel are easily calculated. We assume that the free cadmium concentration inside the gel solution ($[Cd_g^{2+}]$) is equal to that in the bulk solution, after correcting for the water volume fraction and any Donnan partitioning. With this in mind, we have set out to estimate ensuing concentrations of species in the gel phase, be

they dissolved or adsorbed, from measurements of the equilibrium partition/accumulation (c_g^*/c_w^*) of cadmium and humic acid between sample solution and gel phase.

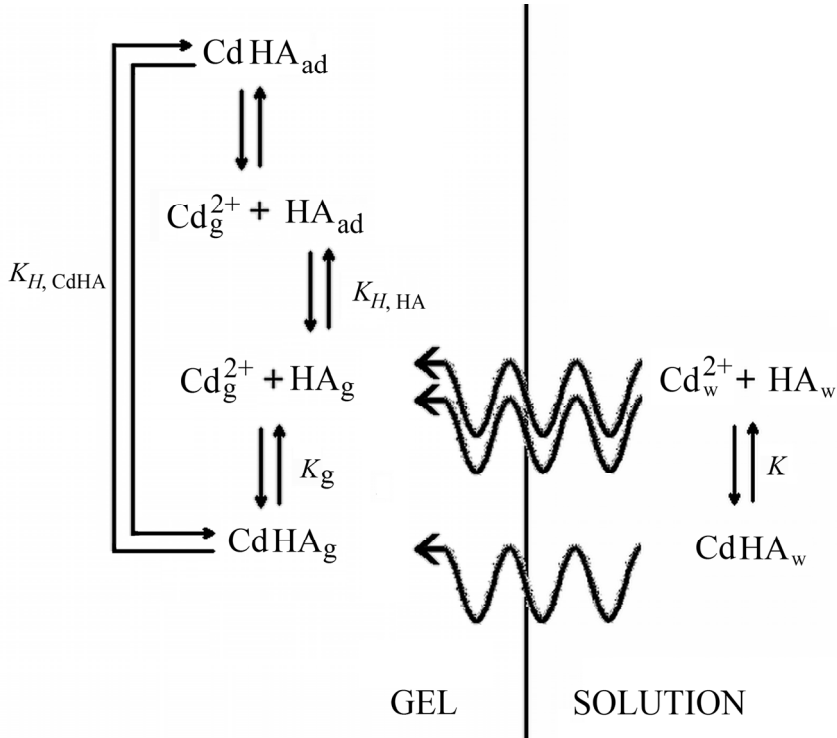


Figure 1. Schematic representation of species and processes involved in the aided transport and accumulation of cadmium in the hydrogel by HA. Subscripts w, g and ad denote species in solution, gel phase and directly adsorbed to the gel backbone, respectively.

5.3. Experimental

5.3.1. Humic and fulvic acids

The humic acid used was a forest soil humic acid (FSHA) (30-32), which has previously been used in gel based sensor experiments. It was originally extracted from forest floor material taken from the Tongbersven forest (Oisterwijk, The Netherlands). Suwannee River Fulvic Acid (SRFA) standard was obtained from IHSS (Cat. No. 1S101F). These compounds have also been previously studied (29-34) providing pH titration data

(34), as well as information on elemental content and concentrations of acidic functional groups, collected in Table 1.

	Elementary Composition (% (w/w))			Acidic Functional Groups (mol kg ⁻¹ C)	
	C	H	O	Carboxyl	Phenolic
FSHA	52.9	5.4	39.3	4.35	7.41
SRFA	52.44	4.31	42.20	11.44	2.91

Table 1. Elemental content and acidic functional groups for purified humic acid from the Tongbersven (FSHA) and Suwannee River fulvic acid standard (SRFA).

5.3.2. Set-up of DET

The gels were prepared and stored until use, according to the protocol for APA-gels provided by DGT Research in Lancaster. They were cast at 0.50 mm thickness (resulting in 0.84 mm effective total gel layer thickness in aqueous medium). The water volume fraction (α) for this gel type (open pore) is 0.95 (20), and this number has been used in correcting all data points for the excluded volume effect. Below 10 mM ionic strength the gel is negatively charged at the pH of the experiments (6.1), causing a slight Donnan partitioning effect in the case of 2 mM ionic strength, which has been corrected for by the corresponding Boltzmann factor (23, 27). DET experiments were carried out with the gel floating freely in the sample medium, enabling diffusion into the gel from both sides, which implies that the effective δ_g is only half the total gel layer thickness.

5.3.3. Other experimental equipment

All glassware used was acid-washed in several steps using HNO₃ (ultrapure, Fluka) and ultra pure water. Spectroscopic measurements were performed on a Hitachi U-2010 UV/Visible spectrophotometer. Elution of metal from the gels was performed with HNO₃ directly in polystyrene tubes standard for ICP-MS trace metal analysis. Data were measured by ICP-MS (Perkin Elmer; Elan 6000).

5.3.4. Experimental setup

Fresh solutions were prepared for each experiment, using 0.001-1 M NaNO₃ (pro analysis, Merck). DET experiments were carried out with HA concentrations in the range of 0.6-10 mg L⁻¹ and FA concentrations in the range of 2.6-50 mg L⁻¹. Although adsorption of trace metals to this gel has been reported (16, 35), we assume the adsorbed form of non-complexed cadmium is insignificant compared to the other metal species. The ranges of

these HA and FA concentrations are of the same order as found in common natural systems, while being sufficient for detection by UV/Vis spectroscopy. Cadmium was added such that $[Cd]/[HA]$ and $[Cd]/[FA]$ ratios were the same for each experiment (25 μmol per gram and 50 μmol per gram, respectively), and sufficient excess ligand conditions were met for the initial stage. MES buffer (from MES sodium salt, Sigma, and HNO_3) was used to keep the pH constant at 6.1. During gel layer deployment the temperature was kept constant at 22 °C and volumes were kept airtight to prevent evaporation over time. Gels deployed came from at least 2 different batches per experiment, with at least 3 gels per batch. Error bars in subsequent results therefore represent standard deviation of all collected data points.

The polyacrylamide gel discs were prepared for UV/Vis measurement directly after extraction from the solutions. After extraction, a square of gel, size 1 cm^2 , was immediately cut out to ensure a good fit into the standard sized UV/Vis cuvette. The cutout was mounted vertically, such that the light beam traverses the gel material perpendicular to the square surface. The wavelength range of the UV/Vis measurements was 244-500 nm. No difference in spectrum characteristics of FA/HA between water and gel phase was observed in this range. The largest UV background signal in this range is caused by NO_3^- , with a relative minimum compared to the FA/HA spectrum at a wavelength of 280 nm. Thus this particular wavelength was selected for the measurements. In cases of significant accumulation, the observed color change was clearly visible to the bare eye.

5.4. Results and discussion

5.4.1. SRFA

The FA is known to be very soluble in water, and is therefore not expected to have a large affinity for the more hydrophobic gel backbone. DET experiments with SRFA in the presence of cadmium showed no accumulation effects; within an experimental error of 10% the $(c_{g,FA}^*/c_{w,FA}^*)_T$ ratio is at or below unity for all FA concentrations (since this partitioning result is trivial, the data is not explicitly included). Due to its deprotonated carboxyl groups, the FA is to some extent excluded from the negatively charged gel phase, resulting in FA concentrations close to the detection limit inside the gel. When comparing these results to the previously determined accumulation of SRFA in the absence of metal (21), this is not unexpected, since in these experiments most of the data points already showed full partition of the FA between gel and aqueous medium. Cadmium in the presence of this FA displayed no enhanced concentrations either, apart from the expected Donnan partitioning.

5.4.2. FSHA

Figure 2a and 2b present the FSHA accumulation data in the presence of cadmium for 11 mM and 2 mM ionic strength, respectively (the complementary accumulation of cadmium is shown in figure 3 and discussed below). The graph shows that, as in the absence of metal, a substantial accumulation of HA takes place ($(c_{g,HA}^*/c_{w,HA}^*)_T \gg 1$). Clearly there is an approximately linear relationship between the HA concentration in the gel and that in the sample medium, with large differences between the accumulation coefficients for the different ionic strengths.

When comparing these results to the accumulation of FSHA in the absence of metal (21) it can be seen that the overall accumulation effect is decreased by a factor of almost 2. As before, the observed accumulation is higher at lower ionic strength. It is assumed that the explanation involving the amphiphilic nature of humic substances and the formation of pseudomicelles still applies in the presence of cadmium. The effect of the lower ionic strength is then that the increased electrostatic repulsion reduces pseudomicelle formation, which in turn leads to an increased affinity of the FSHA for the polymer backbone of the gel. The presence of cadmium does however seem to significantly mitigate this increased electrostatic repulsion, even though the total ionic strength (e.g. 2 mM) does not change notably with the amount of cadmium added to the system (order 0.1 μ M). Therefore it seems that bound metal ions facilitate micellization via reduction of charge and bridging (12). Depending on the characteristics of the HA this may lead to significant changes in size of aggregates (36).

Figure 3 presents the cadmium accumulation data in the presence of FSHA for two ionic strengths. The data unambiguously show that significant cadmium accumulation takes place, even after correcting for Donnan partitioning at 2 mM ionic strength. At first glance, the higher accumulation of cadmium at lower ionic strength seems to be linked to the higher accumulation of FSHA. Cadmium does accumulate to a lower extent than FSHA, which can be largely explained by the preferential adsorption of uncomplexed HA to the gel backbone. Free Cd^{2+} has been shown not to accumulate (22). Hence cadmium is adsorbed to the gel only in the form of its HA complex (figure 1), which is more strongly micellized and therefore has lower affinity than its non-complexed form.

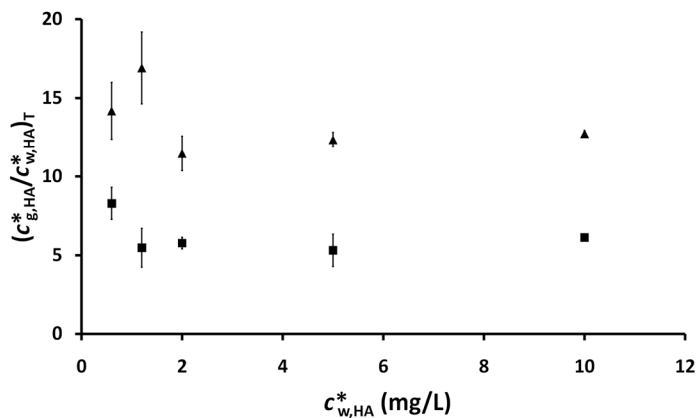


Figure 2a. Normalized FSHA accumulation in DET at ionic strength of 11 mM, in the presence (\blacksquare) and absence (\blacktriangle) of cadmium (absence data from (21)) as a function of bulk FSHA concentration.

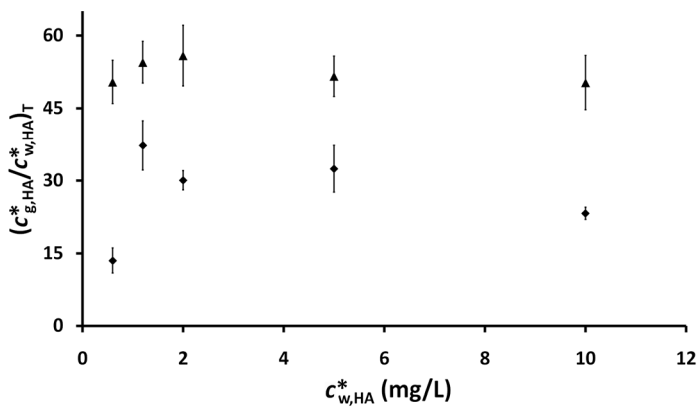


Figure 2b. Normalized FSHA accumulation in DET at ionic strength of 2 mM, in the presence (\blacklozenge) and absence (\blacktriangle) of cadmium (absence data from (21)) as a function of bulk FSHA concentration.

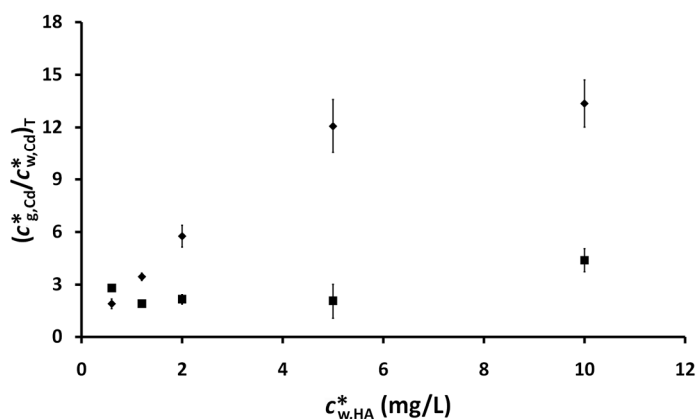


Figure 3. Normalized Cadmium accumulation in DET at ionic strengths of 11 mM (■) and 2 mM (◆) as a function of bulk FSHA concentration. Total concentration ratio of cadmium and HA in the system was constant

I (mM)	2					11				
[FSHA] _T (mg L ⁻¹)	0.6	1.2	2	5	10	0.6	1.2	2	5	10
f_{CdHA_g}	0.001 - 0.01					0.001 - 0.01				
$f_{CdHA_{ad}}$	0.38	0.64	0.89	0.97	0.99	0.81	0.81	0.88	0.94	0.99
$f_{Cd_g^{2+}}$	0.62	0.36	0.11	0.026	0.012	0.19	0.19	0.12	0.060	0.015

Table 2. Fractions of all cadmium species inside the gel phase, calculated from the values represented in Figures 2 and 3, for different experimental conditions. Please note that calculations assume a complexation constant similar to that in solution, and are therefore shown only for the purpose of illustrating trends.

A striking feature of the cadmium accumulation is the difference in slope between the two ionic strength series. For the higher ionic strength case, as with the accumulation of FSHA, there seems to be a linear relationship between the metal concentration in the gel and that in the sample medium. It may be speculated that there is a slight rise in $(c_{g,Cd}^*/c_{w,Cd}^*)_T$, but the experimental data do not provide solid evidence for this. However, at lower ionic strength the $(c_{g,Cd}^*/c_{w,Cd}^*)_T$ ratio does clearly rise with increasing total metal concentration. From the trends in the calculated data (table 2), it seems as if this rise is mainly caused by a significant increase in the contribution of $CdHA_{ad}$ to the sum of all metal species in the gel phase.

Let us now consider the electrostatic properties of the FSHA in some detail. At the given pH and ionic strength, the FSHA has approximately 30 deprotonated carboxyl groups per molecule (34). Given a radius of about 5 nm (31, 38), and assuming that the charged groups are spread evenly over the entire HA volume, the typical distance between two nearest neighbour COO^- groups (l_s) is roughly 3 nm. The Debye length amounts to 3 and 7 nm for 11 mM and 2 mM ionic strength respectively, implying that typically $l_s \approx \kappa^{-1}$ in the 10 millimolar regime. The overall potential distribution is then intermediate between that of local fields around individual charges and that of a fully smeared-out cooperative field (37). As a consequence, the speciation of humics and its metal complexes in the gel phase will generally be very sensitive to ionic strength in the centimolar regime. First of all, the Donnan correction on the HA concentration in the gel solution phase is extremely large. Given the pH (6.1) and the Cd^{2+}/COO^- ratio (1:64), we typically have one Cd^{2+} bound per HA, leading to a net charge per molecule of some 25- in the sample solution. Transfer of this polyanion from the 2 mM ionic strength solution to the gel phase, with a Donnan potential of about -10 mV requires about 20 kJ mol⁻¹. The consequence is that the equilibrium concentrations of the complex CdHA and free HA species, as present in solution, are decreased by a factor of more than 1,000 in the gel phase. Other HA species, more rich in cadmium, are favored by the gel which has a larger $[Cd^{2+}]$ to exploit this. Indeed, the speciation inside the gel is totally different from that in solution: the metal-to-ligand ratio is drastically enhanced and the stability of the Cd/HA complex is correspondingly lowered (4). Systematic studies at various metal-to-ligand ratios, in combination with independent measurements of the Donnan potential in the gel phase, will be mandatory in analyzing the case of large humic acids with tens of charged sites per molecule.

It may be concluded that DET experiments with FSHA and SRFA clearly show that humic substances and their accumulation are significantly influenced by the presence of heavy metal ions, and vice versa. The accumulation of both FSHA and metals is strongest at relatively low ionic strength, implying a very serious accumulation under freshwater conditions. While insufficient screening of charged groups prevents FSHA to micellize, which maximizes its binding by the gel backbone, it is the same charged groups that also withhold FSHA from even entering the gel phase. We recommend that this study is extended to different combinations of humic acids and heavy metals, to see how their accumulation and gel partition properties compare for various ionic strengths.

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Chapter 6

Impact of humic/fulvic acid gel partitioning on dynamic metal speciation by DGT

Pascal L.R. van der Veeken, Herman P. van Leeuwen

Submitted.

Abstract

Metal complexes of humic and fulvic acid species in aquatic and soil media are generally known to be significant. In speciation analysis by DGT (Diffusive Gradient in Thin Film), polyacrylamide hydrogels are commonly used for the diffusive gel layer. Various effects of the presence of humic species on the amount of metal detected by DGT have been observed, but the role of the different metal/humic species is still unknown. Recent DET data showed that humic acid significantly accumulates in the polyacrylamide hydrogel, both in the absence and in the presence of any metal. Here we discuss the impact of this accumulation on the properties of the DGT metal flux, under various experimental conditions.

6.1. Introduction

Over the last decades, research into aquatic metal ion species has made significant progress, notably on the level of dynamic speciation (1-4). Several techniques have been proposed for analyzing the properties of a variety of metal species over a range of timescales, including a suite of sensors based on diffusive gel layers (5, 6). In the latter category, Diffusive Gradients in Thin film (DGT) and Diffusive Equilibration in Thin film (DET) (7-9) are based on diffusion of metal ion species into a gel layer.

The impact of complexing agents such as humic substances on dynamic metal ion speciation in aquatic media is of far reaching significance (3). Such natural macromolecules are highly complex and diverse, and are found in a large variety of environmental media, from soils and sediments to marine waters and freshwaters. Fulvic acids (FA) are smaller in size than humic acids (HA), while the latter generally have lower functional group density (10, 11). Many of these substances, especially the humic acids, also have amphiphilic properties. This is mainly due to the abundance of charged or polar functional groups at the exterior of the HA molecules as compared to the more hydrophobic inner structure. The environmental abundance and ligand traits of humic and fulvic acids, notably the distributed nature of the thermodynamic and kinetic features of their complexes, imply they are also of major importance for the bioavailability of metals (12).

Research into the effects of humics on metal ion transport in DGT speciation sensors is facing uncertainties concerning the gel/sample partition behavior of the HA and FA compounds themselves. Recent experiments (13) indicate that both fulvic and humic acids do in fact enter the gel with little restriction. Humic acid has been shown to not only partition, but even to significantly accumulate, to an extent that strongly depends on the ionic strength of the system. This phenomenon seems to be related to the amphiphilic properties of humic substances which enable them to form micellar secondary structures in the higher ionic strength regime (11, 13). Due to their complexing properties, the concentration of FA/HA substances in the gel phase potentially has a profound impact on metal ion transport in the gel. Accumulation of HA exacerbates this impact, as the speciation inside the gel would then be different from that in the sample medium.

Two different types of polyacrylamide gel are commonly used in DET and DGT. They are generally referred to as the “open pore gel” (APA2-gel) and the “restricted gel” (RG-gel). This paper will focus on the influence of HA and FA on the metal species fluxes in gel layers of the open pore type. Specific structural properties of the gels have not been fully determined yet, though pertaining research is intensifying (14-18). This is not only true for polyacrylamide-based gels; recently the first exploratory fluorescence correlation spectroscopy (FCS) studies were carried out on fluorophores in agarose gels (17-18). Recent work on colloidal complexes (14) showed that the pore size of the polyacrylamide-

based gels is substantially larger than order 10 nm (19). The pore size exceeds the typical sizes of FA and HA, and these complexing agents do indeed enter the gel phase (13). Since FA and HA carry charge, other phenomena such as Donnan effects (15, 20, 21) also play a role in the partitioning of these compounds between gel and sample solutions below 10 mM ionic strength (22). All in all, the various properties of FA/HA will generate a concerted modification of the dynamic speciation of metal ions under DGT measurement conditions. After having investigated the mere sample/gel partition characteristics of FA and HA for different conditions in the absence and presence of metal ions (13, 22), we will now focus on the effects and ramifications of partitioning/accumulation of FA/HA and their metal complexes in DGT experiments with open pore gels.

6.2. Theory

6.2.1. General conditions in DGT

Metal ion analysis by DGT is based on the cumulative flux of all metal species through a diffusive gel layer. Usually, the gel layer is covered by a membrane filter (0.45 μm pore size) and backed by a cation-exchange resin layer, which is completely separated from the bulk solution. The thickness of the gel layer is typically much larger (order 10^{-3} m) than the steady-state diffusion layer in solution (thickness on the order of 10^{-4} m (3)) in order to generate fluxes that are solely governed by diffusion in the gel. According to Fick's first law, the steady-state flux (J) of a species i through the gel layer then depends on its diffusion coefficient in the gel ($D_{i,g}$), the diffusion distance (ideally the gel thickness δ_g) and the difference between the concentration in solution (ideally the bulk concentration c_w^*) and that at the gel/resin interface (c'):

$$J = \frac{D_{i,g}(c_w^* - c')}{\delta_g} \quad (1)$$

Generally it is assumed that the cation-exchange resin is sufficiently strong and has a large excess capacity, so that is essentially zero and eq 1 reduces to

$$J = \frac{D_{i,g}c_w^*}{\delta_g} \quad (2)$$

Prior to attainment of this steady-state flux, there is a transient stage in which the concentration profile of the diffusing species develops towards its final form, ideally a linear one. The time, τ_{ss} , necessary to establish diffusive steady-state is governed by both

the mobility of the diffusing species ($D_{i,g}$) and the geometrical characteristics of the diffusion layer. In its most elementary form, is given by

$$\tau_{ss} = \delta_g^2 / D_{i,g} \quad (3)$$

The DGT deployment time needs to be sufficiently long compared to τ_{ss} , in order to render the impact of the initial transient insignificant. Once a steady state has been reached, the metal transport across the gel can be formulated as

$$J = \frac{1}{A} \left(\frac{\Delta m}{\Delta t} \right)_{ss} \quad (4)$$

where (Δm) is the mass of metal transported during time interval (Δt) through the gel/sample interface (surface area A). The amount of accumulated metal is ultimately determined by ICP-MS, enabling simultaneous measurement of all metals collected. This metal content is then recalculated into the “DGT-labile” concentration in solution, which is the sum of the free and DGT-labile metal species. Such basic DGT calculation requires knowledge on the diffusion coefficients (in the gel) of the relevant complexes and absence of Donnan effects.

6.2.2. Modifications of the diffusive process

When considering a sample solution/dispersion containing only free metal and one type of labile complex under excess ligand conditions, the mean diffusion coefficient (\bar{D}) of such a system is a mobility-weighted average over the free metal M, and the bound metal ML (23):

$$\bar{D} = \frac{(D_M c_M + D_{ML} c_{ML})}{c_T} = D_M c_M \frac{(1 + \varepsilon K')}{c_T} \quad (5)$$

in which

- D_M and D_{ML} are the diffusion coefficients for the free and the bound metal respectively,
- c_T is ($c_M + c_{ML}$)
- $\varepsilon = D_{ML}/D_M$ and $K' = K c_L = c_{ML}/c_M$; $\varepsilon K'$ is a constant only for essentially constant ligand concentrations (i.e. sufficient excess of ligand).

For a given ion/ligand combination, the relative importance of the two diffusion terms in eq 5 is determined by c_{ML}/c_M . Only if this concentration ratio between bound and free metal is well above D_M/D_{ML} (which for humic acids is already well above unity itself), will \bar{D} approach D_{ML} . \bar{D} in the gel, \bar{D}_g , decreases with increasing fraction of complex entering the gel phase.

The effect of \overline{D}_g on the DGT response is straightforward. The ratio between the steady-state flux for the labile metal-ligand complex system and that for the blank (metal only) is

$$\frac{J}{J_{\text{blank}}} = \frac{\overline{D}_g c_{T,g,l} / \delta_g}{D_{M,g} c_T / \delta_g} = \frac{c_{T,g,l}}{c_T} \frac{(1 + \varepsilon K'_g)}{(1 + K'_g)} \quad (6)$$

in which $c_{T,g,l}$ refers to a complex species that both penetrates the gel and is DGT-labile. Partial permeation by the ligand species will result in a K'_g lower than K' in the sample. The overall change in \overline{D}_g will also have a significant effect on the time needed to reach a steady-state (see eq 3). E.g. for metal/humic complexes with a D_g on the order of $10^{-10} \text{ m}^2 \text{ s}^{-1}$ under conditions of $\varepsilon K' > 1$, τ_{ss} will be a factor of 10 higher as compared to the blank humic-free case. For the common gel layer thickness of *ca.* 1 mm, establishment of steady state diffusion will then require some 10 hours. Accumulation of the free ligand and complex species further complicates estimation of τ_{ss} .

6.2.3. Donnan reduction/enhancement

The polyacrylamide gel layer used in DGT has a thickness δ_g on the order of 1 mm (7), hence for all ionic strengths δ_g is typically much larger than the electrostatic screening length (Debye length) κ^{-1} . When the gel possesses fixed charged groups at a density such that the average charge-charge separation is not much larger than κ^{-1} (see 24 for details), a Donnan potential difference, Ψ_D , will develop between the bulk gel environment and the bulk electrolyte solution. The equilibrium Donnan potential is given by (25):

$$\Psi_D = \frac{RT}{zF} \operatorname{arcsinh}\left(\frac{z_g N}{2zc}\right) \quad (7)$$

where $z_+ = z_- = z$ is the charge number of the (symmetrical) supporting electrolyte of bulk concentration c , z_g is the charge number of the fixed charges with concentration N , R is the gas constant, T the absolute temperature, and F the Faraday. A finite Donnan potential has electrostatic consequences for the partitioning of all ions between the gel phase and the electrolyte solution. The equilibrium Donnan partitioning coefficient, Π_D , is defined as (20):

$$\Pi_D = \frac{c_g}{c_w^*} = \exp(-z_i F \Psi_D / RT) \quad (8)$$

where c_g represents the concentration within the gel of an ion of charge z_i and bulk solution concentration c^* . In case of a slightly anionic gel, such as the polyacrylamide gel used in DGT and DET, the equilibrium concentration of all positive species will be enhanced within the gel relative to the bulk solution, whereas all negative species will have reduced concentrations within the gel phase.

Three examples of possible situations with linear concentration profiles across the gel layer are represented in Figure 1, with two extremes shown for each; inert complexes that are not involved in the measured flux (dashed horizontal profile) and fully labile complexes ($c' = 0$). Conventional DGT conditions described earlier are represented here by case 1. Since the polymer network of the gel reduces the free volume available for diffusion, the effective D_g may be expected to be somewhat lower than D_w (26, 27). For the gels used in commercially available DGT, D_g/D_w values for simple ions range from 0.8 to almost unity (27), depending on the nature and concentration of the cross-linker in the polymer network. This is mainly due to excluded volume as expressed by the water volume fraction (α) of these gels instead of an actual reduction in mobility, and will not be discussed here.

Case 2 represents significantly reduced concentrations of species in the gel. Causes may vary, but a primary example is Donnan partitioning, i.e. the electrostatic lowering of the equilibrium concentration of negatively charged species due to the negative Ψ_D of the gel. On the other hand, the concentration of the free metal ion and its positive complex species will in that case be enhanced (not made explicit in Figure 1). Another example is the exclusion of complexes by size (14). For the APA gels, the profile of the relatively small and mobile free ion remains essentially unaffected. The consequences of the size exclusion for the DGT flux are deduced from eq 6. Contributions from large gel-permeating complexes will be masked by their lower diffusion rates (compare $\varepsilon K'_g \approx 1$ in eq 6). For $\varepsilon K'_g \gg 1$, complexes are so strong that in spite of their reduced mobility they will dominate over the free metal term in the overall diffusion flux. When analyzing DGT data for such systems, the assumption of impermeability towards large complexes leads to overestimation of the free metal concentration.

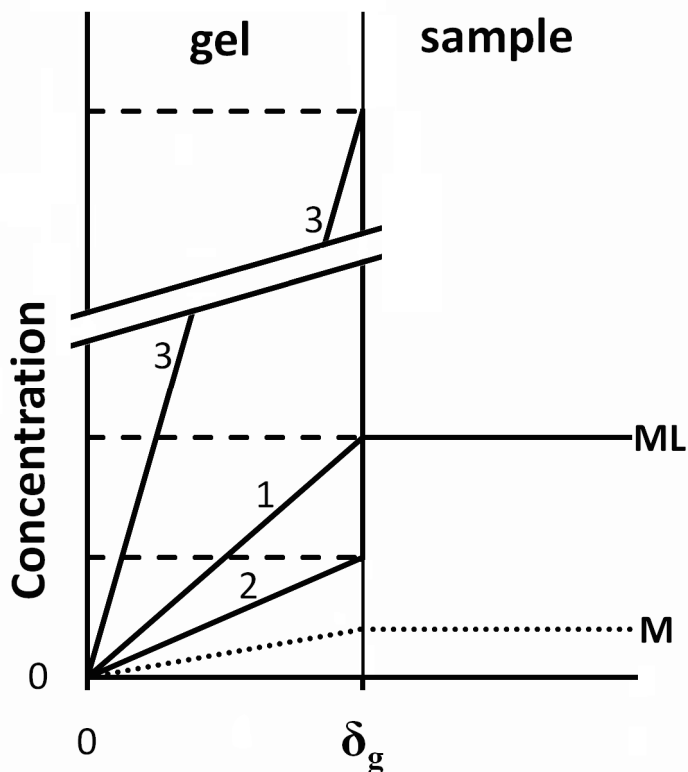


Figure 1. Sketch of the steady-state concentration profile across a diffusive gel with thickness δ_g , for both labile (solid lines) and inert (dashed lines) versions of three different cases of complexed metal. Cases include (1) basic DGT conditions, (2) reduced ML in the gel phase and (3) enhanced ML in the gel phase. Concentrations of free metal and fully labile complexes reach zero at the resin/gel interface ($c' = 0$, far left). Inert concentrations remain virtually constant throughout the diffusive gel layer. Excluded volume effects and the solution diffusion boundary layer have been omitted.

Case 3 illustrates the reverse situation of case 2, namely an enhancement of complexed metal in the gel. Again, Donnan partitioning may be the cause if the complex has a net positive charge, as may occur with neutral ligands. Another possibility could be an affinity of the ligand for the gel backbone, as in the case of humics. As pictured by Figure 1/case 3, the total complex concentration in the gel layer then outweighs that in the sample solution. For humics and freshwater samples, the gel/water concentration ratio has been found to reach the order of 10 to 100. In the case of backbone affinity the total

accumulation is not necessarily representative for the impact, since complexes have zero mobility when adsorbed. However, a large amount of complex in the gel solution results in a tremendous impact on the eventual steady state DGT flux, as well as on τ_{ss} (which would come to several days).

6.3. Experimental

6.3.1. Humic and fulvic acids

The humic acid used was a forest soil humic acid (FSHA) (29-31), which has previously been used in gel based sensor experiments. It was originally extracted from forest floor material taken from the Tongbersven forest (Oisterwijk, The Netherlands). Suwannee River Fulvic Acid (SRFA) standard was obtained from IHSS (Cat. No. 1S101F). These compounds have also been previously studied (29-34) providing pH titration data (34), as well as information on elemental content and concentrations of acidic functional groups, collected in Table 1. Bulk cadmium species concentrations (Table 2) were calculated with Visual MINTEQ (ver. 2.61), using the specific NICA-Donnan parameters for SRFA (35) and FSHA (36).

	Elementary Composition (%(w/w))			Acidic Functional Groups (mol kg ⁻¹ C)	
	C	H	O	Carboxyl	Phenolic
SRFA	52.44	4.31	42.20	11.44	2.91
FSHA	52.9	5.4	39.3	4.35	7.41

Table 1. Elemental content and acidic functional groups for purified humic acid from the Tongbersven (FSHA) and Suwannee River fulvic acid standard (SRFA).

	I (M)	DGT signal	interpretations		NICA-Donnan calculation
		J/J_{blank}	$(c_{\text{DGT}} = c_{\text{M,w}}^*)$ $c_{\text{M,w}}^*/c_{\text{T}}^*$	$(c_{\text{DGT}} \Rightarrow \bar{D} \Rightarrow c_{\text{M,w}}^*)$ $c_{\text{M,w}}^*/c_{\text{T}}^*$	$c_{\text{M,w}}^*/c_{\text{T}}^*$
SRFA	0.011	0.55±0.04	0.55	0.15	0.16
	0.002	0.51±0.06	0.51	0.19	0.23
FSHA	0.011	0.75±0.09	0.75	0.74	0.66
	0.002	0.93±0.08	0.93	0.89	0.75

Table 2. Measured DGT flux ratio for different experimental conditions, compared to experimental signal interpretations. FA and HA bulk concentrations were 10 and 2 mg L⁻¹ respectively. NICA-Donnan derived values for $c_{\text{M,w}}^*/c_{\text{T}}^*$ were calculated using the specific parameters for SRFA (35) and FSHA (36) at the experimental pH (6.1). $[\text{Cd}_{\text{T}}^{2+}]$ for SRFA and FSHA experiments were 50 and 10 nM respectively.

6.3.2. Set-up of DGT

Both diffusion layer and resin gels were homemade and stored until use, according to the protocol provided by DGT Research in Lancaster. The diffusion gels were cast at 0.50 mm thickness (resulting in 0.84 mm effective total gel layer thickness in aqueous medium). The water volume fraction (α) for this gel type (open pore) is 0.95 (19), and this number has been used in correcting all data points for the excluded volume effect. Below 10 mM ionic strength the gel is negatively charged at the pH of the experiments (6.1), causing a slight Donnan partitioning effect in the case of 2 mM ionic strength, which has been corrected for by the corresponding Boltzmann correction in eq 8 for the various ionic species (20, 21).

6.3.3. Experimental setup

All glassware was given a multi-step acid-wash prior to use to ensure no metal contaminations using HNO₃ (ultrapure, Fluka) and ultra pure water. Fresh solutions were prepared for each experiment, using 1-10 mM NaNO₃ (pro analysis, Merck). DET experiments were carried out with a HA concentration of 2 mg L⁻¹ and an FA concentration of 10 mg L⁻¹. These concentrations of HA and FA are of the same order as found in common natural systems, as well as in the range used in previous DET measurements on FSHA/SRFA (13, 22). Cadmium was added such that [Cd]/[HA] and [Cd]/[FA] ratios in the test solution remained constant (around 1:300 on the basis of functional groups), and sufficient excess ligand conditions were met for the initial stage. MES buffer (from MES sodium salt, Sigma, and HNO₃) was used to keep the pH constant at 6.1. During deployment the temperature is kept constant at 22 °C and volumes are kept airtight to prevent evaporation over time. Gels were extracted after a deployment time of 72 hours. Elution of metal from the gels was performed with HNO₃ directly in polystyrene tubes fit for ICP-MS trace metal analysis (Perkin Elmer; Elan 6000).

6.4. Results and discussion

It should be clear that the different cases discussed in the Theory section may each apply to a variable extent from one sample to another. For illustrative purposes, we present straightforward DGT measurements of cadmium in the presence of fulvic and humic acids. The partition behavior of these natural complexants with respect to the polyacrylamide APA2 gel has been discussed in previous papers on their DET characteristics, both in absence and in presence of cadmium (13, 22). The basic complexity of the system containing metal ions is sketched in Figure 2. Here we will discuss the various pitfalls in the interpretation of DGT data on such a natural complex system for which knowledge on the properties of the sample ligands is usually limited.

6.4.1. Fulvic acids

The measured values of the DGT flux ratio for the Cd(II)/SRFA system are presented in Table 2. The most basic interpretation would be to directly relate the measured signal to the free metal ion in solution (i.e. $c_{\text{DGT}} = c_{\text{M,w}}^*$), while using eq 2 with predetermined $D_{\text{M,g}}$ values to calculate this concentration (7). In the presence of significant proportions of labile MFA complexes with diffusion coefficients a factor of 3 to 5 lower than $D_{\text{M,g}}$, the free metal concentration would be overestimated by this approach. However, in early DGT development it was already realized that reasonably small complexes may freely enter the gel, while more recent research shows the sizes of permeating species can be up to the order of 100 nm diameter (14). Indeed, recent DET work shows that both FA and HA (>20,000 Dalton (30)) do permeate the gel phase (13, 22).

Comparing results from NICA-Donnan modelling with DGT-data for several natural water samples led to the conclusion that fulvic and humic metal complexes contribute to the DGT flux by several tens of a percent (37). Many DGT studies have therefore advocated independent investigation of the mobility and lability properties of the complexing ligands in solution. For the present work with SRFA/FSHA, we have used their estimated diffusion coefficients (38, 13) and known NICA-Donnan binding parameters (35, 36). More specifically, we may determine from this information the appropriate ε and K' values for use in eq 6. It is known that FA complexes have no specific interactions with the gel material (13), are free to permeate the entire gel, and are fully labile (39) on the timescale of diffusion across this gel diffusion layer (implying that $K' = K'_g$ and $c_{\text{T,g,l}}/c_{\text{T}} = 1$, with concentration profiles similar to our theoretical case 1). It can be seen from eq 6 that we may then directly compare the flux ratio $(1 + \varepsilon K')/(1 + K')$ with the experimentally

measured DGT signal (J/J_{blank}). As shown in Table 2, the SRFA results are adequately explained by this interpretation, with agreement almost completely within the experimental error. This result concurs with the DET observation that FA fully enters the gel phase but does not accumulate therein.

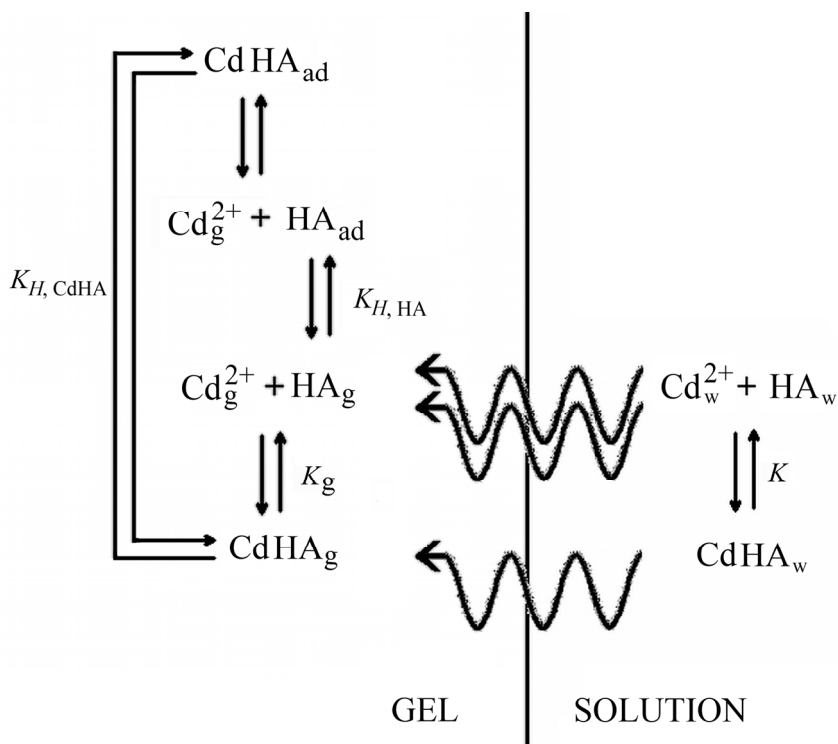


Figure 2. Schematic representation of species and processes involved in the aided transport and accumulation of cadmium in the hydrogel by HA. Subscripts w, g and ad denote species in solution, gel phase and directly adsorbed to the gel backbone, respectively.

6.4.2. Humic acids

Recent DET measurements of FSHA show that affinity of the hydrophobic parts of the FSHA towards the polyacrylamide gel results in substantial accumulation. The extent of accumulation seems to be related to the amphiphilic nature of humic acids, which makes them capable of forming micelle-like structures (10, 11, 40). Since these humic micelles consist of a number of unidentical entities, they are usually referred to as ‘pseudomicelles’. The precise formation properties of humic pseudomicelles are very sensitive to the

physicochemical conditions in the medium, as well as to the type of humic acid. If the ionic strength is so low that electrostatic repulsion between the entities prevents aggregation, the hydrophobic regions are more free to adsorb to the polymer backbone of the gel, resulting in an increase in affinity of FSHA for the gel with decreasing ionic strength. At first sight the HA adsorbed to the backbone of the gel, as well as any metal bound to it, does not directly contribute to the DGT flux. This can be illustrated with eq 5, for the general situation with three different metal species (M, ML and adsorbed ML). The mobility of any substance adsorbed to the gel backbone is practically zero, effectively eliminating its contribution to the flux as long as this is of a steady-state nature. Looking at Figure 1, and ignoring adsorbed species, we can regard the relevant concentration profiles as depicted in case 2, with ML being the dissolved CdHA in the gel solution ($CdHA_g$) which is in local equilibrium with adsorbed species ($CdHA_{ad}$). Thus for labile CdHA with a D_w of 2×10^{-11} to $8 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ (13) the measured flux ratio results in $c_{M,w}^*/c_T^*$ values of 0.74 and 0.89, which is significantly higher than the NICA-Donnan bulk values of 0.66 and 0.75. This shows that this simple assumption of taking $CdHA_g$ equal to $CdHA_w$ (times excluding volume factor) leads us to an overestimation of the free metal concentration.

It is well-known that at lower ionic strengths, the negative Donnan potential of the gel itself has significant consequences for the partitioning of all charged species between the gel phase and the solution. This is particularly relevant for the relatively large FSHA which at the pH of 6.1 carries some 30 negative charges per molecule (22). At first sight the negative charge of the FSHA might be expected to cause a huge reduction of FSHA inside the gel (as in case 2), whereas an enhancement is actually observed. To what extent various humic acid species would really be excluded, and to what extent they modify the charge of the gel layer, would remain unknown without pertaining investigation. DET has shown that HA does equilibrate within about one week (13), and it couldn't do so if it were virtually incapable of entering the gel phase. For the case of FSHA, the combined DET/DGT data suggest that the observed enhanced concentration in the gel is strongly dominated by adsorbed species. A Donnan-modified humic concentration inside the gel alters the speciation of any metal with which it forms complexes. Simultaneously, the increase in negative charge due to accumulated HA will further enhance Ψ_D . The extent of this enhancement follows from comparison between the actual charge density of the gel itself at the given pH and that of the adsorbed HA species. The former is approximately 1.6 mol m^{-3} and the latter, for the case of the highest accumulation factor (around 30), comes to $0.1 \text{ mol charges per m}^3$. Although this change is relatively mild in this particular example, it is significant enough to require consideration for any given combination of hydrogel and humic.

Since all humic compounds are distributed in size and lability, HA species with higher metal loading are expected to more easily accumulate against a negative Donnan

potential and the transport of the metal might even be enhanced. Although the exact distribution of species inside the gel is far from defined, the enhanced flux of metal in the presence of FSHA in Table 2 seems to support this hypothesis. In fact, the flux seems more enhanced at lower ionic strength, when the preference for cadmium-rich CdHA species is stronger. Due to the extraordinary electrostatic nature of the HA/gel combination, the speciation of both the humics and their metal complexes in the gel phase will be very sensitive to ionic strength, especially in the regime encountered in freshwaters. Primary causes for enhanced metal species concentrations in the gel phase are the Donnan enhancement of the free metal and the accumulation of metal in the form of adsorbed complexes. Standard DGT flux evaluations/computations may then easily result in a “DGT-labile concentration” close to or even exceeding the actual total metal concentration. In passing it should be realized that in case of accumulation of the HA, the diffusive steady-state is generally not reached within a day. DGT fluxes determined according to conventional protocol then are still of an undefined transient nature and underestimate the real steady-state flux. The effectively different speciation inside the gel can also cause a violation of the excess ligand condition as valid in the sample. Even without counting the adsorbed FSHA, the metal-to-ligand ratio in the gel is drastically enhanced, and the stability of the Cd/FSHA complex correspondingly lowered (3). It may seem fortunate that this leads to dominance of the diffusion of the free metal ion ($\varepsilon K' \ll 1$), but a careful Donnan correction of the concentration of all diffusing metal species would still be mandatory.

It may be concluded that the sound interpretation of DGT fluxes in not too-well defined natural ligand systems generally requires independent information on parameters such as physicochemical characterization of complexing ligands and gel backbone. Accordingly, the translation of DGT fluxes to metal speciation dynamics in freshwaters containing humic substances may be rather involved and requires careful consideration of factors such as lability, interaction with the gel backbone and Donnan effects. Amongst the recommended strategies are variation of deployment time for accurate determination of the steady-state achievement time, and the deliberate combination of DGT flux measurements with DET equilibrium data on the gel/sample partitioning of both metals and humics.

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Chapter 7

Summary and General Discussion

7.1. Summary and General Discussion

This thesis deals with several aspects of the use of Diffusive Gradient in Thin film (DGT) and Diffusive Equilibration in Thin film (DET) in dynamic metal speciation analysis. It has a clear focus on the properties of the diffusive gel layer, and their possible impact on metal speciation measurements in samples containing nanoparticulate and colloidal complexing agents.

The introductory chapter already announced the paramount feature of the DGT technique, which is the well-defined control over the diffusive layer thickness. DGT realizes this via the introduction of a hydrogel layer in which diffusion is the sole mode of transport for ions and complexes that are able to enter the gel phase. The rate of diffusive transport across the gel layer is then governed by the species mobility and the layer thickness, with the latter parameter being under full control of the analyst.

In order to maintain a limiting steady-state flux across the gel layer, it is backed by a strong cation exchange layer that acts as a perfect sink for metal ions. The most common set-up uses a Chelex-resin. Current research is exploring the use of dedicated binding agents for more specific uses in a variety of environmental systems, e.g. Teasdale et al. (1-4). Nowadays, DGT is being employed for systems that are increasingly complex, and sometimes largely uncharacterized. For example, numerous experiments have been performed on the use of DGT in soils (5, 6), laser ablation for element detection at high spatial resolution (7, 8) and array-type setups for simultaneous deployment of larger numbers of DGT sensor units. Although this creative branching opens up a lot of opportunities, it also introduces a host of potential complications that require dedicated solutions. Nevertheless, more detailed knowledge is required to address the pitfalls that are already present in the most straightforward application of DGT in aquatic systems. Here we do this to a significant extent by combining DGT with DET measurements on the same sample system, using exactly the same gel in each case, to fully utilize the complementary nature of the two techniques.

Chapter 2 investigates the pore size of two common types of DGT diffusive gels. They are usually known as “open pore” (APA2) and “restricted” (RG) gel. Both gels have been shown to be fully permeable to metal ions and small complexes. However, it has been generally assumed that colloidal particles with radii from tens to hundreds of nm are excluded from the gel. This chapter describes DGT and DET measurements in model dispersions of differently sized synthetic particles. These particles bind Pb(II) from solution, in essence providing us with probes using Pb(II) as a marker which is easily detectable by ICP-MS. The particles involved are monodisperse, carboxylated latex nanospheres with radii 40.5 nm and 129 nm. Extensive information on their diffusion coefficients and ion binding affinities for various metals, under a range of pH and ionic

strength conditions, is available. Apart from them being well characterized and excellently monodisperse, the main interest in using these particular particles as model colloidal ligands is their size and their relatively large metal binding site density.

DET results show a very clear difference compared to those for a system where no permeation would take place. Time-resolved investigation of the DET process clearly shows the slow process of diffusion of large particles into the gel, and the amount measured at equilibrium is the sum of the concentrations of the free and particle-bound metal in the gel. DGT, on the other hand, measures the mobility-weighted sum of these concentrations. As a consequence, DGT speciation analysis of samples possibly containing colloidal species call for parallel DET experiments. This combination of techniques makes it possible to determine the concentration of both the free and the particle-bound metal, provided the ratio between the mobilities of the two types of species is known.

In *Chapter 3*, attention is shifted from pore size features to the charge properties of the diffusive gel. Often the assumption is made that the gel matrix is effectively uncharged and chemically inert with respect to the species of interest. Prior to the work performed for this thesis however, research on the same gel (DGT) by Yezek et al. (9, 10) showed significant non-ideal behavior at relatively low ionic strength, suggesting a finite structural electric charge within the gel matrix. Such structural charge within the gel layer gives rise to species concentrations in the gel phase that are different from those in the sample. The steady-state diffusion of ions through the gel is also influenced by the structural charge, but the net effect will depend on the details of the speciation. Perturbations of the concentrations and fluxes of ions in the gel phase as compared to the sample solution are particularly troublesome because quantitative interpretations for submillimolar ionic strength solutions are common in freshwater research. We highlight how to calculate the correction factor needed for the particular ionic strength of the sample and the characteristics of the gel used. However, Donnan partitioning affects not only the total metal concentration in the gel phase, but also the relative concentrations of all charged species. Hence, the entire metal speciation within the gel phase depends on the magnitude of the Donnan potential. Consequently, the relationship between the amount of metal measured by DGT and the corresponding metal species distribution in the bulk sample solution may adopt a rather involved nature. Again, due to the inherent differences between DET and DGT, complementary DET deployment appears helpful, if not indispensable.

Chapter 4 starts our investigation into the effects of the natural ligands humic acid (HA) and fulvic acid (FA) on DGT and DET metal speciation measurements. Knowledge on the impact of such complexing agents on dynamic metal ion speciation is fundamental to understanding many processes in aquatic media. Humic and fulvic acids are highly complex and diverse, and are found in a large variety of natural settings, from soils and sediments to marine waters and freshwaters. The environmental abundance and ligand traits of HA and FA imply that they are also of major importance for the bioavailability of heavy metals.

Previous DGT research has focused on the effects of HA and FA in the sample solutions while ignoring the ultimate sample/gel partitioning fate of the HA and FA particles themselves. Here we analyze solutions of Suwannee River Fulvic Acid standard (SRFA) and a specific Forest Soil Humic Acid (FSHA) and show with DET that these natural complexing agents do enter the gel layer. The humic acid even appears to accumulate in the gel, with typical enrichment factors up to several tens. The accumulation is especially strong at relatively low ionic strength, implying a very substantial accumulation under freshwater conditions, while media with an ionic strength similar to that of marine conditions showed no accumulation at all. Other research on humic acids has shown that, due to the heterogeneous distribution of the charged groups within the humic structure, these substances are able to form so-called “pseudomicelles”. At lower ionic strength, the pseudomicelle formation is counteracted by increased repulsion between the charged groups, which enhances the availability of the more hydrophobic center of the FSHA entity to binding by the polyacrylamide gel backbone instead. Time-dependent DET measurements confirm the migration of humic acid into the gel phase. The rate of this process indicates that the steady-state achievement time for DGT, which is already large due to the low mobilities, will be substantially further enlarged due to the strong accumulation in the gel. Further research on the accumulation properties of different types of humic acids, for various ionic strengths, from freshwaters to marine levels, will be mandatory. The results herein have significant consequences for the interpretation of DGT-data on metal fluxes from aquatic media containing humics and fulvics.

Chapter 5 extends the research to the accumulation of humic and fulvic acids in the presence of metal ions, specifically cadmium(II), and the consequences for interpretation of speciation. The changes in the partitioning of HA and FA, as well as the partitioning of cadmium itself, are measured. At millimolar ionic strength level, DET data show significant accumulation of Cd(II) in the gel phase, on top of some Donnan enrichment. It is shown that the accumulation of HA is significantly influenced by the presence of the cadmium ions, and vice versa. The accumulation of both FSHA and metals is again strongest at relatively low ionic strength. It seems that the presence of metal ions significantly influences the formation of HA pseudomicelles, which in turn has a significant effect on the transport of the metal species themselves through the gel layer. Future research may include an extension of this study to different combinations of humic acids and heavy metals, and their accumulation and gel partition properties for various ionic strengths.

Chapter 6 discusses the impact of the accumulation of humic acid in the gel layer on the properties of the DGT metal flux for various experimental conditions. The inherent complications due to the accumulation of the different species are exacerbated by the simultaneous Donnan effects for all charged species. For samples with insufficiently defined natural ligands, the interpretation of DGT fluxes generally requires independent information on physicochemical parameters such as affinities between complexing ligands

and the gel backbone. Accordingly, the translation of DGT fluxes to metal speciation dynamics in freshwaters containing humic substances may be rather involved and certainly requires careful consideration of factors such as lability, interaction with the gel backbone and Donnan effects. Therefore it is strongly recommended to (i) perform measurements including variation of deployment time for accurate determination of the steady-state achievement time, and (ii) deliberately combine DGT flux measurements with DET equilibrium data on the gel/sample partitioning of both metals and humics.

7.2. Outlook

From the preceding overview it can be seen that all sections of this work advocate the combined use of DGT and DET. Though the precise reasons differ depending on the chapter's subject, the foundations are identical.

Firstly, the signals measured by the two techniques complement each other. DGT is a flux method, and the signal therefore depends on the mobility and lability of the species involved. Though the ability of different species to permeate the gel is also relevant in DGT, it is the primary feature of the measured signal in DET. In basic DET deployment, one waits for equilibrium to be achieved, rendering mobility and lability immaterial for the end result. The mere discriminator here is size, which takes us to the properties of the hydrogel. Although the two techniques have significantly different principles, there is no barrier in making their measurement device spatially identical. It is fairly easy to use one and the same hydrogel for both DGT diffusive gel and DET equilibration gel, which obviously facilitates their combined application. Commercially available DGT deployment units already used in most research can easily mount a gel for DET instead, making simultaneous deployment of both techniques under the same sample conditions very convenient. The amount of effort needed for the inclusion of DET in a DGT deployment is ultimately insignificant considering the gain in information on the partition properties of the sample species.

As for the drawbacks of DET, it is true that for practically metal-free solutions the accumulated metal content of DGT is much easier to determine than the equilibrium concentration of DET. However, dedicated labs and the further lowering of the detection limit of current techniques such as ICP-MS may overrule that drawback of DET in a matter of years. As for the time needed to achieve DET equilibrium, it is clear from previous chapters that the steady-state achievement time in DGT can easily turn out to be significantly longer than expected. Therefore it may be appropriate to re-evaluate deployment times in terms of their representing steady state diffusion conditions.

Finally, as for the polyacrylamide gels, developers should not forget to keep an eye on new practical applications of previously undeveloped gels. Gels in current use have been tested for suitability and reproducibility, originally and mainly by Davison et al. (11, 12). Even taking into account the parameters discussed in this thesis, such as Donnan effects, swelling effects and chemical bonding of sample molecules, this has resulted in the use of the most suitable gels available at the time. This means that, looking at the stability and robustness of chemical gels at that stage, this research focused mainly on chemical gels. However, physical gels are gaining more and more interest and become increasingly well-defined. An example is in the class of associative telechelic polymers. Basically, these are polymers consisting of three distinct blocks, of which the end blocks form supramolecular knots as physical cross-links. These can for example be hydrophobic blocks that end-cap a hydrophilic middle section (13, 14). Quantitative modeling has been hampered mainly by the polydispersity of both the chains and the physical cross-links. However, quite recently different polypeptide chains have been produced which are monodisperse and have a very well defined cross-link formation (15). The gels are also remarkably stable, and are expected to maintain their stability in water of temperatures below 20 °C for at least the typical duration of a DGT measurement, i.e. several days. More importantly, these chains are produced with yeast strains, a technique which provides a large amount of control over the precise sequence of polypeptides, opening the way to a significant range of possibilities and fine-tuning of the gel properties such as pore size, structural charge, hydrophobicity, etc.. Whether these gels will ultimately be suitable for use in DGT remains to be seen, but they certainly deserve the attention of DGT researchers.

7.3. References

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Samenvatting

Dit proefschrift behandelt verscheidene aspecten van het gebruik van de technieken "Diffusive Gradient in Thin film" (DGT) en "Diffusive Equilibration in Thin film" (DET) in dynamische metaalspeciatieanalyse. In metaalspeciatieanalyse probeert men te achterhalen in welke vormen (speciës) metalen aanwezig zijn in een systeem, b.v. als vrij ion of gebonden aan andere deeltjes (gecomplexeerd). Deze speciatie is vooral interessant voor de biobeschikbaarheid, een mate voor hoe makkelijk de verschillende speciës van (vaak giftige) metalen opgenomen worden door levende organismen. DGT en DET zijn beide technieken die zijn gebaseerd op diffusiekenmerken van metaalspeciës in een gellaag, enerzijds met constante diffusie door de gellaag heen, door het bepalen van de hoeveelheid opgehoopt metaal *achter* de gellaag na een vastgestelde tijd (DGT), anderzijds door het meten van de hoeveelheid metaal *in* de gellaag nadat er een evenwicht is bereikt tussen de gellaag en het monster (DET).

Er is in dit proefschrift een duidelijke focus op de kenmerken van de diffusie in de gellaag, en op de mogelijke effecten van deze kenmerken bij metaalspeciatiemetingen in monsters die complexerende componenten van uiteenlopende afmetingen bevatten. Zoals vermeld in het inleidende hoofdstuk 1, is beheersing van de dikte van de diffusielag de belangrijkste eigenschap van de DGT-techniek. Deze controle wordt bereikt door de introductie van de gellaag, waarin diffusie de enige manier van transport is voor ionen en complexen die de gellaag kunnen binnendringen. De snelheid van transport van metaalspeciës door de gellaag wordt bepaald door hun mobiliteit en door de vrij te kiezen laagdikte.

Om een limiterend en constant transport door de gellaag te handhaven, brengt men achter de gellaag een laag met kationenwisselaar aan die metaalionen sterk bindt. Voor specifieke situaties zijn ook andere bindingsmaterialen beschikbaar. Tegenwoordig wordt DGT meer en meer toegepast op steeds ingewikkelder, soms grotendeels ongekarakteriseerde systemen, zoals DGT in grondbodems. Toepassingen met lasertechnieken voor elementendetectie in hoge resolutie en specifiek gegroepede opstellingen van DGT sensoren bieden nieuwe perspectieven. Ondertussen verdienen de basale kenmerken van DGT nog de volle aandacht. In dit kader onderzoeken wij hier o.a. de doorlaatbaarheid en de wisselwerking tussen speciës en gel. Daarbij wordt herhaaldelijk gebruik gemaakt van de combinatie van DGT met DET voor hetzelfde monster, om zo veel mogelijk te profiteren van de complementariteit van de twee technieken.

In *Hoofdstuk 2* wordt de doorlaatbaarheid van twee typische DGT diffusiegelen beschreven. Deze staan bekend als "open pore" en "restricted" gel. Het is bekend dat beide gelen volledig doorlaatbaar zijn voor metaalionen en kleine complexen. Er werd voorheen veelal aangenomen dat kolloïdale deeltjes ter grootte van tientallen tot honderden

nanometers de gel niet binnendringen. Dit hoofdstuk beschrijft DGT en DET metingen in modelmonsters die deeltjes met verschillende afmetingen bevatten. De gekozen deeltjes zijn bolvormige, gecarboxyleerde latexbolletjes met stralen van 40.5 nm en 129 nm. Deze deeltjes binden metaalionen die vervolgens eenvoudig te detecteren zijn met ICP-MS (Inductively Coupled Plasma Mass Spectrometry), een techniek die vaak wordt gebruikt om lage concentraties metaalionen te bepalen. De diffusie- en bindingseigenschappen van deze deeltjes, voor verscheidene metalen en relevante waarden van pH en ionsterkte, zijn onafhankelijk bepaald in voorafgaand onderzoek.

Het volgen van het DET evenwichtsproces toont duidelijk een langzame diffusie van de relatief grote latexdeeltjes, en de evenwichtshoeveelheid van metaal in de gel is de som van het vrije en het gebonden metaal. DGT daarentegen meet de som gewogen op basis van mobiliteit van deze concentraties. Het ligt daarom voor de hand om DGT-metingen in monsters met deeltjesgebonden metaal uit te breiden met DET experimenten. Deze combinatie maakt het mogelijk om zowel de vrije als de gebonden metaalconcentraties te bepalen, mits de mobiliteit van de deeltjes bekend is.

In *Hoofdstuk 3* wordt aandacht besteed aan de ladingseigenschappen van de diffusiegel. Er wordt vaak aangenomen dat de gelmatrix zo goed als ongeladen is en chemisch inert ten opzichte van de te meten deeltjes. Eerder werk van Yezek et al. heeft echter aangetoond dat er significante meetafwijkingen zijn bij betrekkelijk lage ionsterkte, wat een structurele elektrische lading in de gel suggereert. Deze afwijkingen zijn met name problematisch in de analyse van zoet water. We behandelen hier een correctieprocedure. De structurele elektrische lading zal echter de relatieve concentraties van *alle* geladen deeltjes veranderen (Donnanverrijking/verarming), en zo de gehele metaalspeciatie in de gelfase beïnvloeden. Het gecompliceerde verband tussen de DGT-meting en de speciatie in het monster zal daarom significant kunnen worden verhelderd met behulp van DET metingen.

Hoofdstuk 4 gaat in op de effecten van natuurlijke liganden zoals humuszuren (HA) en fulvinezuren (FA) op DGT en DET metaalspeciatie-metingen. Voldoende kennis van deze complexerende stoffen met betrekking tot dynamische metaalspeciatie is fundamenteel voor het begrijpen van vele processen in waterige media. Humus- en fulvinezuren zijn erg complex en divers, en worden aangetroffen in vele verschillende systemen, van bodems en zandgronden tot zoet- en zoutwatersystemen. De uitgebreide aanwezigheid in de natuur en de ligandeigenschappen van HA en FA suggereren dat deze stoffen ook van uitermate groot belang zijn voor de biobeschikbaarheid van metalen. In dit hoofdstuk bespreken we de analyse van monsters met Suwannee River Fulvic Acid standard (SRFA) en een specifiek Forest Soil Humic Acid (FSHA) en tonen met DET aan dat deze natuurlijke complexerende stoffen de gellaag ingaan. Het FSHA lijkt zelfs te accumuleren in de gel, tot concentraties die tientallen malen groter kunnen zijn dan in het watermonster. De accumulatie neemt toe met afnemende ionsterkte, hetgeen wijst op een sterke ophoping in zoet water. Bij ionsterktes zoals die voorkomen in zeewater treedt geen accumulatie op. Dankzij de

heterogene distributie van de geladen groepen in de humusstructuur, zijn humuszuren in staat om zogenaamde “pseudomicellen” (enigszins georganiseerde clusters van humuszuren) te vormen. Bij lagere ionsterkte wordt pseudomicelvorming tegengegaan door de grotere afstoting tussen de geladen groepen. Dan is het hydrofobe centrum vrij om een binding aan te gaan met de polyacrylamide ruggengraat van de gellaag. Tijdsafhankelijke DET-metingen bevestigen de migratie van HA naar het binnenste van de gellaag. De snelheid van dit proces geeft aan dat de tijd die vereist is om met DGT een stationaire diffusiesituatie te bereiken niet alleen lang is door de lage mobiliteit van het HA, maar ook wordt verlengd door de sterke accumulatie van HA in de gellaag. Verder onderzoek naar de accumulatie-eigenschappen van verschillende typen HA, bij ionsterktes variërend van zoet- tot zoutwaterconcentraties, is vereist. De resultaten zijn essentieel voor de kwantitatieve interpretatie van DGT-data voor watermonsters die HA en/of FA bevatten.

Hoofdstuk 5 breidt het onderzoek uit naar de accumulatie van HA en FA in de aanwezigheid van metaalionen zoals cadmium(II) en de gevolgen voor de speciatieanalyse. De veranderingen in de verdeling van HA en FA over monster en gel, alsook die van de cadmium, werden gemeten. Bij ionsterkte in de orde van millimolen is met DET een significante accumulatie van Cd(II) in de gel te zien, bovenop de Donnanverrijking. De accumulatie van HA wordt significant beïnvloed door de aanwezigheid van de cadmiumionen, en vice versa. De accumulatie van FSHA en de metaalionen is wederom het grootst bij lage ionsterkte. Het lijkt erop dat de aanwezigheid van metaalionen een significant effect heeft op de vorming van pseudomicellen, die op hun beurt het transport van de metaalionen door de gellaag beïnvloeden. Toekomstig onderzoek zou deze experimenten kunnen uitbreiden naar het bepalen van de accumulatie en de verdeling over gel en monster voor verschillende combinaties van humuszuren en zware metalen.

Hoofdstuk 6 bespreekt de invloed van HA-accumulatie in de gellaag op de eigenschappen van het transportproces van metaal in DGT onder verschillende experimentele omstandigheden. De complicaties veroorzaakt door de accumulatie van de verschillende speciës worden verergerd door de Donnaneffecten die door de ladingen van individuele speciës bepaald worden. Voor monsters met onvoldoende gedefinieerde natuurlijke liganden vereist de interpretatie van de DGT fluxen in het algemeen onafhankelijke informatie over fysisch-chemische parameters zoals de affiniteiten van complexerende liganden voor de gelmatrix. Het verband tussen DGT-fluxen en de dynamische metaalspeciatie in zoet water kan daardoor uitermate ingewikkeld zijn. Er dient dan ook grondig rekening gehouden te worden met factoren zoals labiliteit, wisselwerking met de gel en Donnaneffecten. Het verdient aanbeveling om (i) metingen te doen bij variërende bemonsteringsduur om het bereiken van een stationaire diffusiesituatie zeker te stellen, en (ii) doelbewust DGT transportgegevens te combineren met DET evenwichtsgegevens voor de verdeling van zowel metalen als humuszuren over gel en monster.

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Pascal

Levensloop

Pascal Leon René van der Veecken werd in 1980 op 30 november geboren te Terneuzen. Hij spendeerde zijn eerste jaren in het dorp Zandstraat, om daarna te verhuizen naar Poonhaven. In 1999 behaalde hij zijn VWO gymnasium diploma aan de Jansenius Scholengemeenschap in Hulst, en begon vervolgens aan de studie Moleculaire Wetenschappen aan de Wageningen Universiteit. Voor zijn stage keerde hij terug naar Zeeuws-Vlaanderen, om precies te zijn de afdeling microscopie bij Dow Chemical R&D in Terneuzen. Zijn eerste afstudeervak bij de vakgroep Agrofysica was een volledig theoretisch literatuuronderzoek over quantummechanica. Voor zijn tweede afstudeervak werkte hij bij de vakgroep Biofysica aan fotoakoestische spectroscopie. In oktober 2005 begon hij aan zijn promotieonderzoek bij de vakgroep Fysische chemie en kolloïdkunde, waarvan het resultaat in dit boekje te bekijken valt. Op dit moment is hij bezig aan een postdoc onderwerp bij dezelfde vakgroep voor DSM.



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- o Metal Speciation by DGT, DGT Conference, 8 October 2009, Cagliari, Italy

SENSE Coordinator PhD Education and Research

Mr. Johan Feenstra

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