Effect of Humic Acid on Metal Uptake Measured by Diffusive Gradients in Thin Films Technique

[a]B. DOČEKAL, [b]V. ŘEZÁČOVÁ-SMETKOVÁ*, and [b]H. DOČEKALOVÁ

[a]*Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, CZ-611 42 Brno, Czech Republic*
e-mail: docekal@iach.cz

[b]*Institute of Chemistry and Technology of Environmental Protection, Brno University of Technology,*
CZ-612 00 Brno, Czech Republic
e-mail: rezacova@fch.vutbr.cz

Impact of the presence of humic acid (HA) on the metal uptake of cadmium, copper, nickel, and lead in the diffusive gradients in thin films technique (DGT) was studied. Both the diffusive and resin gel were composed of the agarose polyacrylamide hydrogel, the resin one also with embedded Chelex-100 ion exchanger. A restrict gel, excluding high-molecular species, was used in complementary experiments. Metal concentrations of 50 μg dm^{-3} of Cd, Cu, Ni, and Pb, various mass concentrations of HA substance of ρ/(mg dm^{-3}): 0, 0.3, 1, 3.1, 10, 31, 100, and 316, and concentration of 0.05 mol dm^{-3} sodium nitrate were adjusted in experimental solutions (pH = 6.8). The DGT probes were deployed in solutions at (26 ± 2)°C for up to 32 h. After elution, the overall DGT-accumulated masses of metals were determined by electrothermal atomic absorption spectrometry and apparent diffusion coefficients of individual metals were evaluated. The HA substance at approximately equimolar (1 mg dm^{-3}) and higher concentrations with respect to metals ions considerably reduces the DGT-measured metals uptake. This suppression effect is more pronounced at higher concentrations of HA substance, it is element-specific and increases in the sequence Cd, Ni, Pb, Cu. HA species diffuse through the permeable gel and affect predominantly the interaction of metal ions with specific iminodiacetic groups of the resin by competitive reactions. This is documented by a very good agreement of the experimental data and the data obtained for the theoretical model of the effect of HA on the conditional equilibrium of metal-iminodiacetic group. Reduced mobility of larger molecular species of metal—humic acid complexes plays a minor role.

Humic acids (HA) are the most widespread complexing ligands. They occur in soils, sediments, and waters as a product of the chemical and biological transformation of animal and plant residues. As HA play important role in the environmental chemistry, they have great importance with regard to the quality and the retention of metals and pollutants in the environment. The mechanisms involved in interactions are not clear and may vary depending on the physico-chemical properties of the compounds, pH, and redox status, as well as on the form of HA in environmental systems. HA have ability to affect considerably mobility and availability of metals in the environment.

Trace metals such as Cu, Mn, Ni, and Zn play important role as essential elements in the environmental systems. Increased concentration of these elements or elements of anthropogenic origin such as Cd and Pb may be toxic. Biological responses of organisms are often related to the concentration of different metal species and properties of the environment.

The *in situ* techniques are only able to provide reliable measurement of the metals species, because they overcome serious problems related to system changes due to sampling and additional sample treatments. Recently, the technique of diffusive gradients in thin films (DGT) was developed [1, 2]. This technique is capable of measuring average *in situ* concentration of labile metal species in aqueous systems [3—6]. Its application *in situ* in soil and sediment allows to determine overall resupply fluxes of mobile metals species [7—9], to measure metal profiles in pore water at the sediment—water interface [10—12] and to assess availability of metals [13—16].

In DGT, two types of polyacrylamide hydrogel are used. The layer of specific ion-exchange resin (Chelex-100) embedded in a hydrogel is overlaid by a further ion-permeable layer, *i.e.* diffusive hydrogel of thickness Δg. Transport of metal ions, labile and mobile metal species from the sampled system (external solution) to the resin gel is determined by the concentration gradi-

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*The author to whom the correspondence should be addressed.

ent in the ion-permeable gel. After diffusion through the layer of ion-permeable gel, metals are immobilized in the resin gel (sink), so that the concentration in the gel solution approaches zero at the interface of both gels. Provided the resin sink is not saturated and the concentration in the external solution remains constant, a linear concentration gradient is established in the ion-permeable gel. The flux (F) of metal ions from the external solution to the resin gel can be calculated by Fick's first law of diffusion

$$F = D \cdot \rho / \Delta g \qquad (1)$$

where ρ is the mass concentration of metal in the external solution and D is the diffusion coefficient of a metal in the diffusive gel. The flux is also defined as the mass of the metal (m) transported through the diffusion area (A) during the deployment time (t)

$$F = m/(A \cdot t) \qquad (2)$$

The metal flux and subsequently the mean metal concentration in the external solution can be calculated from the mass of the metal accumulated in the resin gel (m). Metal content is usually determined after elution of the resin gel by a suitable analytical method (atomic absorption spectrometry, inductively coupled plasma atomic emission and mass spectrometry, *etc.*).

The aim of this paper is to report on the influence of humic acid present in the solution on the metal uptake measured by the DGT probe, on estimation of an impact of HA on the response of the DGT technique in real systems.

EXPERIMENTAL

Gels and DGT Probes

Both diffusive and resin gels were based on an agarose polyacrylamide hydrogel (APA-gel). This hydrogel was composed of 15 vol. % acrylamide (Boehringer) and 0.3 vol. % patented agarose-derived cross-linker (DGT Research, Lancaster, UK). Freshly prepared ammonium persulfate initiator (70 mm³ of 10 mass % solution) and TEMED (tetramethylethylenediamine) catalyst (20 mm³) were added to each 10 cm³ of gel solution. In addition, the resin gel solution contained also 2 g of Chelex-100 ion exchanger (Na-saturated, "200—400 mesh", Bio-Rad) in the same volume.

The gel solution was cast immediately after mixing between two glass plates separated by a spacer foil of thickness of 0.5 mm and 0.25 mm for preparation of diffusive and resin gels, respectively. The gels were set in an incubator at $(42 \pm 3)\,°C$ for 45 min. Less amount of initiator and catalyst solutions were used to prolong the setting process of resin gels in order to allow the
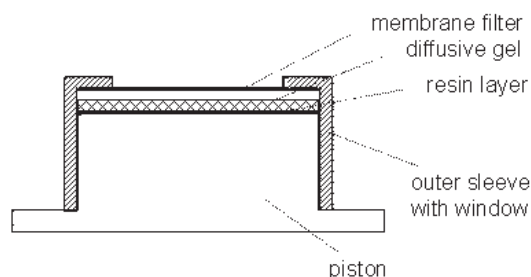

Fig. 1. Scheme of the DGT sampling probe.

resin to settle by gravity to one side of the gel. The prepared gels were then hydrated in high-purity water for at least one day to allow them to swell to a stable thickness of 0.8 mm for the diffusive gel and 0.4 mm for the resin gel, respectively. A fully hydrated diffusive gel contained about 95 mass % of water.

Gel discs of 25 mm in diameter were cut from the gel layer by a plastic tool (DGT Research). Discs of diffusive and resin gels were stored in 0.01 mol dm⁻³ high-purity sodium nitrate (anal. grade, Lachema, Brno, Czech Republic) solution and in water, respectively.

For complementary experiments, a restrict gel (RG-gel, purchased from DGT Research), was used instead of the diffusive APA-gel. This type of disc excludes by size diffusion of high molecular species. Thus, only low molecular species can diffuse through pores in size below 1 nm and can reach the resin disc [3].

The DGT unit (DGT Research) based on a simple polypropylene tight-fitting piston design (see Fig. 1) was employed in experiments. It consisted of a backing cylinder (piston) and a front cap (sleeve) with a 20 mm diameter exposition window. A disc of resin gel was placed on top of the backing cylinder oriented with the resin beads upwards, and then a disc of diffusive gel was placed directly on top of it. Before assembling the DGT unit, the diffusive gel was overlaid by the 130 μm thick, 0.45 μm pore size Gelman Laboratory Supor®-450 hydrophilic polyethersulfone membrane filter (Pall Corporation, Michigan, USA) to protect the gel against damage.

Procedure

A portion of humic acid substance (Product No. 53680, Fluka, Switzerland) was dissolved in water with addition of minimum 2 % NaOH solution to obtain finally 1 g dm⁻³ stock solution. Standard stock solutions of metals (1 mg cm⁻³) were of Astasol® grade (Analytika, Prague, Czech Republic), sodium nitrate was of anal. grade (Lachema, Brno, Czech Republic), and nitric acid was of Suprapur grade (Merck, Darmstadt, Germany). In all experiments, high-purity

water (MilliQ, Millipore) was used. For deployment experiments, metal solutions were prepared by diluting stock solutions to obtain metal concentration of 50 μg dm^{-3} of Cd, Cu, Ni, and Pb each of them, various concentrations of humic acid substance of $\rho/(\text{mg dm}^{-3})$: 0, 0.3, 1, 3.1, 10, 31, 100, and 316 and 0.05 mol dm^{-3} sodium nitrate. pH of solutions was adjusted to 6.5—7.0 by adding diluted nitric acid. Final volume of each solution was 2 dm^3. Before application, the solutions were equilibrated under intensive mixing for 24 h.

The DGT probes were deployed in solutions in duplicate at (26 ± 2)°C for 8 h, 24 h, and 32 h. Portions of 10 cm^3 solutions were taken before and after deployment of DGT units and the samples were stabilized in cleaned polypropylene vials with 10 mm^3 of concentrated nitric acid for subsequent analysis.

After deployment, the DGT units were rinsed with high-purity water before being disassembled. The disc of resin gel was retrieved, placed in an acid-cleaned plastic vial, and accumulated metals in the resin were eluted by immersing the disc in 1 cm^3 of high purity 1 mol dm^{-3} nitric acid for 24 h. Afterwards, analytes (Cd, Cu, Ni, Pb) were determined in eluate and exposition solutions by electrothermal atomic absorption spectrometry employing Perkin—Elmer Model 4110 Zeeman atomic absorption spectrometer (ET AAS). Recommended conditions and standard addition method of calibration were applied.

The overall DGT-accumulated masses of metals were calculated from their concentrations in eluate solutions. Total volume of the gel and eluate 1.16 cm^3 (for 0.4 mm thick resin disc) and elution efficiency of 0.8 were taken into account in the evaluation. Assessment of these values was described elsewhere [2, 17]. Mean fluxes of metals in the DGT device and the concentration of metals in the external solution were obtained by using eqns (2) and (1), respectively. Simultaneously, blank experiments were carried out and considered in the evaluation.

RESULTS AND DISCUSSION

The DGT units were deployed in solutions of Cd, Cu, Ni, Pb, and HA for up to 32 h. For example, the results are shown in Fig. 2 for uptake of copper. Other metals exhibited the same behaviour. The mass of metal (m) accumulated during the deployment time (t) was converted to normalized metal uptake (m/ρ). This normalization obtained by combining eqns (1) and (2) allowed compensation for different initial metal concentrations in individual external solutions found after the equilibration period. Stability of spiked pH-neutral metal solutions increased with increasing concentration of humic acid, probably due to complexation of metal ions by humic acid ligands. Concentrations of all elements in individual solutions, established after the equilibration procedure,
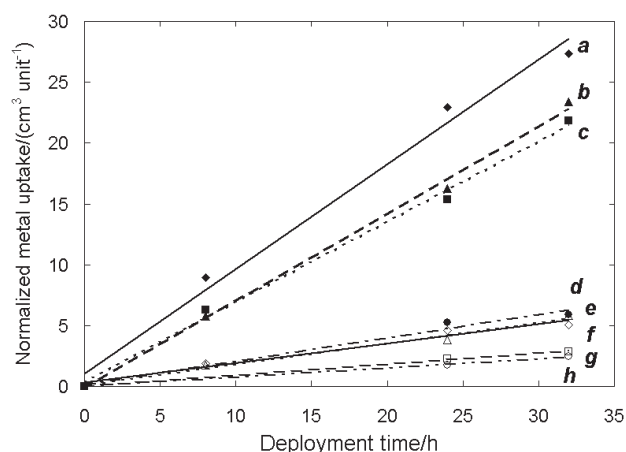
Fig. 2. Time dependence of accumulated mass of copper during deployment, converted to normalized uptake of copper (m/ρ) at a temperature of 26°C for various humic acid concentrations ($\rho_{\text{HA}}/(\text{mg dm}^{-3})$): a) ♦ 0; b) ▲ 0.3; c) ■ 1; d) ● 3.1; e) ◇ 10; f) △ 31; g) □ 100; h) ○ 316.

did not significantly change within an experimental uncertainty of 10 % (RSD) during the subsequent deployment period.

The mass of the accumulated metal, the normalized metal uptake (m/ρ), increases linearly with time for all of the concentrations of HA in solutions. This indicates that the concentration gradients as well as the flux of DGT-attainable metal species are constant, time-independent, during the whole deployment period of 32 h. However, the metal flux is reduced, when HA are present in solutions, the metal uptake decreases with increasing concentration of HA. This observation could be explained by reduced mobility of metal-ion complexes related to high-molecular HA species or by formation of relatively stable complexes of HA with metal ions.

Combining eqns (1) and (2), diffusion coefficients (D) can be calculated when taking into account diffusion area ($A = 3.14$ cm^2) and thickness of the diffusive layer (sum of gel and membrane filter thickness $\Delta g = 0.94$ mm). Those diffusion coefficients can be considered as apparent diffusion coefficients (D_{app}) of the metal under specific conditions (conditional equilibrium). Their values represent the metal fluxes per unit deployment time, unit diffusion area, unit metal concentration in the external solution, and unit thickness of the diffusive gel layer.

The values of apparent diffusion coefficients (D_{app}) can be compared with literature data (D_{tab}) related to diffusion of simple metal ions in a diffusive gel (DGT Research) at a temperature of 26°C: 6.26×10^{-6} cm^2 s^{-1} for Cd, 6.40×10^{-6} cm^2 s^{-1} for Cu, 5.93×10^{-6} cm^2 s^{-1} for Ni, and 8.25×10^{-6} cm^2 s^{-1} for Pb [18], so that the ratio of both apparent and reported values of diffusion coefficients ($D_{\text{app}}/D_{\text{tab}}$) results in a normalized value. These normalized data are compared in Fig. 3 for all metal
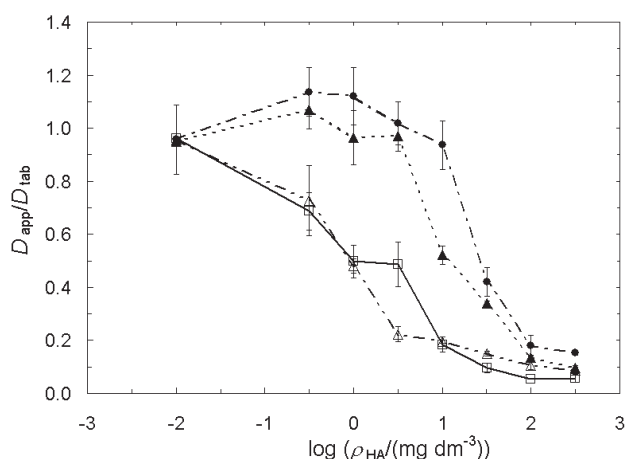
Fig. 3. Influence of humic acid concentration ($\rho_{HA}/(\text{mg dm}^{-3})$) on the normalized values of the apparent diffusion coefficients (D_{app}/D_{tab}) for: ● Cd, △ Cu, ▲ Ni, □ Pb (temperature 26 °C).

ions and humic acid concentrations. The values decrease when increasing concentration of humic acid substance. The dependences bend at humic acid concentration of approximately 1 mg dm^{-3}. When taking into account an average relative molar mass of the HA substance of 600—1000 g mol^{-1} (according to information in the product sheet), one can conclude that HA affect the metal uptake very close to equimolar ligand—metal ratio n(ligand) : n(metal) $\approx$ 1 in the solution.

It can be assumed that molar excess of HA compounds over metal ions is established for HA concentration higher than 3 mg dm^{-3}. *Zhang* and *Davison* showed [19] that the diffusion coefficient of HA is not significantly affected by anchoring metals. Diffusion coefficients published in literature [19—23] reached values in the range of $(0.5$—$4.0) \times 10^{-6}$ cm^2 s^{-1}, depending on the origin of various HA substances. Our complementary experiments with the restrict gel (RG-gel), which excludes diffusion of high-molecular species, showed the same dependences for APA- and RG-gels. Furthermore, the same concentration of a metal in the external solution and in the diffusive gel was found after 60 min deployment period in experiments when diffusive gel was only used (resin gel was not inserted in the DGT probe). Humic acid species present in the permeable gel would restrict very fast diffusion of other metal-containing species into the resin gel, especially when large excess of HA is applied. However, no depletion of the metal uptake was observed for a long period as can be concluded from Fig. 2. Furthermore, element nonspecific behaviour should be observed when mobility of metals is reduced due to formation of complexes with HA. However, this is not the case as documented in Fig. 3, in which element-specific influence of HA can be observed. These observations suggest that humic acid

complexes diffuse through the permeable gel, and thus affect the interaction of metal ions with specific groups of the resin.

The results of application of the DGT probes in solutions of metals accompanied with humic acid in different concentrations can be explained by conditional equilibrium of complex formation. The interaction of metal ions with the specific iminodiacetic groups of the resin (Chelex-100) can be affected by competitive reactions in solutions and in the resin gel. Formation of metal—HA complexes and protonation of the functional group of resin (iminodiacetic acid) are the side reactions to the bonding of metal ions. The effect of side reactions is usually quantified by the side reaction coefficient α.

Generally, immobilization of metal ions (Me) in the resin (R) by iminodiacetic groups can be expressed

$$\text{Me(aq)} + \text{R(s)} \leftrightarrow \text{MeR(s)} \qquad (A)$$

Stability of the MeR complex is characterized by the stability constant β_{MeR}

$$\beta_{MeR} = \frac{[\text{MeR}]}{[\text{Me}]\,[\text{R}]} \qquad (3)$$

where [Me], [R], and [MeR] are the equilibrium concentrations of reactants and the product under standard conditions. When considering side reactions, the conditional stability constant β'_{MeR} can be expressed

$$\beta'_{MeR} = \frac{[\text{MeR}']}{[\text{Me}']\,[\text{R}']} = \frac{\beta_{MeR}}{\alpha_{Me(HA)} \cdot \alpha_{R(H)}} \qquad (4)$$

where [Me′], [R′], and [MeR′] are the conditional concentrations of respective species in the equilibrium, and $\alpha_{Me(HA)}$, $\alpha_{R(H)}$ are the individual side reaction coefficients. The side reaction coefficient $\alpha_{Me(HA)}$ represents the effect of metal complexation with HA

$$\text{Me(aq)} + \text{HA(aq)} \leftrightarrow \text{Me—HA(aq)} \qquad (B)$$
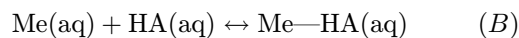
and is defined

$$\alpha_{Me(HA)} = 1 + \beta'_{Me-HA}\,[\text{HA}'] = 1 + \frac{\beta_{Me-HA} \cdot c_{HA}}{\alpha_{HA(H)}} \qquad (5)$$

where β'_{Me-HA}, β_{Me-HA}, c_{HA}, and [HA′] are the conditional stability constant, the stability constant of humic acid—metal complex Me—HA, the total concentration, and the conditional equilibrium concentration of HA, respectively. The side reaction coefficient $\alpha_{HA(H)}$ defines the protonation of HA. It is expressed

$$\alpha_{HA(H)} = 1 + \beta_{HA}\,[\text{H}] = 1 + \frac{[\text{H}]}{K_{aHA}} \qquad (6)$$

where K_{aHA} is the dissociation constant of acidic groups of HA and [H] is the concentration of hydrogen

Table 1. Summary of Values of Constants of Stability of Metal—Resin (β_{MeR}) and Metal—Humic Acid (β_{MeHA}) Complexes and Dissociation Constants of Resin (K_{aRH}) and Humic Acid (K_{aHA}) Included in Calculation of the Side Reaction Coefficients (pH = 6.8, Relative Molecular Mass $M_{\mathrm{r}} = 1000$)

Resin	Value	Humic acid	Value
$\log \beta_{\mathrm{CdR}}$	5.73	$\log \beta_{\mathrm{CdHA}}$	4.90
$\log \beta_{\mathrm{CuR}}$	10.6	$\log \beta_{\mathrm{CuHA}}$	6.40
$\log \beta_{\mathrm{NiR}}$	8.13	$\log \beta_{\mathrm{NiHA}}$	5.30
$\log \beta_{\mathrm{PbR}}$	7.45	$\log \beta_{\mathrm{PbHA}}$	6.20
$\mathrm{p}K_{\mathrm{aRH}}$	2.98	$\mathrm{p}K_{\mathrm{aHA}}$	4.86

ions in the solution. The protonation of the specific iminodiacetic groups in the resin R can be expressed

$$\mathrm{H^+(aq) + R^-(s) \leftrightarrow HR(s)} \qquad (C)$$

The side reaction coefficient $\alpha_{\mathrm{R(H)}}$ is then defined

$$\alpha_{\mathrm{R(H)}} = 1 + \beta_{\mathrm{RH}}[\mathrm{H}] = 1 + \frac{[\mathrm{H}]}{K_{\mathrm{aRH}}} \qquad (7)$$

where K_{aRH} is the dissociation constant of the functional group of the resin and [H] is the concentration of hydrogen ions in the solution.

Above expressions allow to quantify the total side reaction coefficient α, composed by a product of partial coefficients ($\alpha_{\mathrm{Me(HA)}} \cdot \alpha_{\mathrm{R(HA)}}$), and to calculate the conditional stability constant of the complex Me—R, β'_{MeR}. The values of constants K_{aRH} and β_{MeR} found in aqueous solutions [24] were chosen as the best surrogate for values of constants of heterogeneous resin/solution equilibrium. Many studies were focused on determination of K_{aHA} and $\beta_{\mathrm{Me-HA}}$ [25—38]. However, a wide range of the data can be found that relate to different HA substances, e.g. for $\mathrm{p}K_{\mathrm{aHA}}$ values of 3.4—5.5 and for $\log \beta_{\mathrm{Cu-HA}}$ values of 3.5—8.8 were reported. Some papers presented also sequences of stability of Me—HA complexes, e.g. stability of complexes increases in the order Cu, Pb, Ni, Cr, Cd in Refs. [34, 35], Cu, Pb, Ni, Cd in Refs. [36, 37] or Cu, Cd, Ni, Zn in Ref. [38]. Obviously, the best estimate for these constants $\mathrm{p}K_{\mathrm{a}} = 4.86$ and $\log \beta_{\mathrm{Me-HA}}$ for complexes Cd—HA and Pb—HA of 4.9 ± 0.4 and 6.2 ± 0.7, respectively, can be obtained from the works of *Lubal et al.* and *Fetch* in Refs. [30—32]. These reported values relate to the same HA substance (Fluka, Product No. 53680). For Cu and Ni, the sequence of stability of Me—HA complexes presented in Ref. [30] was taken into account in assessing values of $\log \beta_{\mathrm{Me-HA}}$. The total concentration of humic acid c_{HA} and the acidity (pH) of the solution [H] were known experimentally, and relative molar mass of HA (M_{r}) of 1000 g mol^{-1} was taken from Ref. [39]. The individual values of constants used in evaluation are summarized in Table 1.
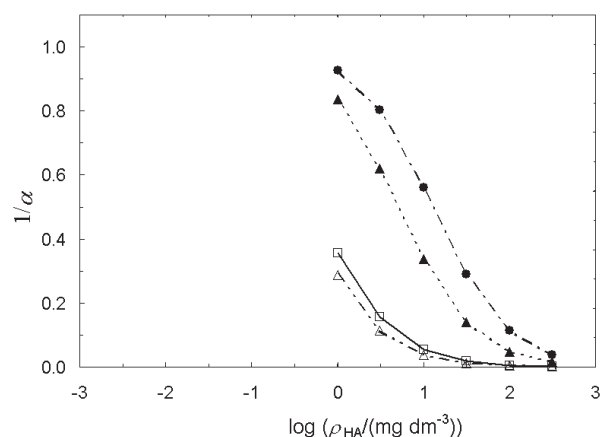

Fig. 4. Dependence of reciprocal values of side reaction coefficient $1/\alpha$ on varying humic acid concentration ($\rho_{\mathrm{HA}}/(\mathrm{mg\ dm^{-3}})$) in solutions for: ● Cd, △ Cu, ▲ Ni, □ Pb.

The total side reaction coefficient α allows quantifying the influence of humic acid substance. The reciprocal value of this coefficient ($\alpha \geq 1$) represents according to eqns (4) and (5) the ratio of stability constants $\beta'_{\mathrm{MeR}}/\beta_{\mathrm{MeR}}$, i.e. the effect of HA in the interaction of metal ions with specific iminodiacetic groups of resin. Consequently, values $1/\alpha$ obtained for various concentrations of HA can be compared directly with the ratio of diffusion coefficients ($D_{\mathrm{app}}/D_{\mathrm{tab}}$) presented in Fig. 3. The data calculated from the model of conditional complex equilibria are plotted for four studied elements in the graph in Fig. 4 for comparison sake. It should be emphasized that contribution of acid-base equilibria is negligible ($\alpha_{\mathrm{R(H)}} = 1.0002$ and $\alpha_{\mathrm{HA(H)}} = 1.0115$) at pH of 6.8 applied in these experiments. One can recognize the same trend in dependences, including element-specific behaviour, when comparing graphs in Figs. 3 and 4. Obviously, competitive complexation reactions of HA with metal ions in the aqueous solution play decisive role in affecting DGT response that is reflected by the reduced metal uptake. Reduced mobility of larger molecular species of metal—HA complexes plays evidently a minor role in this instance.

Potentially, DGT technique can be employed in characterization of environmental systems, in fractionation/speciation studies. Modifying the specific groups of the resin in terms of weak/strong ligands, one can control the selectivity of the technique, affinity to certain metal species. In order to shed light on processes and mechanisms of influence of HA in assessing metals species in environmental systems by DGT technique, further investigations should be carried out with various types of humic acid substances, as well as various functional groups anchored to the resin.

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