Helmholtz-Zentrum Dresden-Rossendorf (HZDR)

HELMHOLTZ ZENTRUM
DRESDEN ROSSENDORF

Dynamics of metal-humate complexation equilibria as revealed by isotope exchange studies – a matter of concentration and time

Lippold, H.; Eidner, S.; Kumke, M. U.; Lippmann-Pipke, J.;

Originally published:

October 2016

Geochimica et Cosmochimica Acta 197(2017), 62-70

DOI: https://doi.org/10.1016/j.gca.2016.10.019

Perma-Link to Publication Repository of HZDR:

https://www.hzdr.de/publications/Publ-22647

Release of the secondary publication
on the basis of the German Copyright Law § 38 Section 4.

CC BY-NC-ND

Accepted manuscript, in press for Geochim. Cosmochim. Acta 2016
http://dx.doi.org/10.1016/j.gca.2016.10.019

Dynamics of metal-humate complexation equilibria as revealed by isotope exchange studies – a matter of concentration and time

Holger Lippold [a,*], Sascha Eidner [b], Michael U. Kumke [b], Johanna Lippmann-Pipke [a]

[a] *Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, Permoserstr. 15, 04318 Leipzig, Germany*

[b] *University of Potsdam, Institute of Chemistry (Physical Chemistry), Karl-Liebknecht-Str. 24-25, 14476 Golm, Germany*

* Corresponding author.

E-mail address: h.lippold@hzdr.de (H. Lippold).

Abstract

Complexation with dissolved humic matter can be crucial in controlling the mobility of toxic or radioactive contaminant metals. For speciation and transport modelling, a dynamic equilibrium process is commonly assumed, where association and dissociation run permanently. This is, however, questionable in view of reported observations of a growing resistance to dissociation over time. In this study, the isotope exchange principle was employed to gain direct insight into the dynamics of the complexation equilibrium, including kinetic inertisation phenomena.

Terbium(III), an analogue of trivalent actinides, was used as a representative of higher-valent metals. Isotherms of binding to (flocculated) humic acid, determined by means of ^{160}Tb as a radiotracer, were found to be identical regardless of whether the radioisotope was introduced together with the bulk of stable ^{159}Tb or subsequently after pre-equilibration for up to 3 months. Consequently, there is a permanent exchange of free and humic-bound Tb since all available binding sites are occupied in the plateau region of the isotherm. The existence of a dynamic equilibrium was thus evidenced. There was no indication of an inertisation under these experimental conditions.

If the small amount of ^{160}Tb was introduced prior to saturation with ^{159}Tb, the expected partial desorption of ^{160}Tb occurred at much lower rates than observed for the equilibration process in the reverse procedure. In addition, the rates decreased with time of pre-equilibration. Inertisation phenomena are thus confined to the stronger sites of humic molecules (occupied at low metal concentrations). Analysing the time-dependent course of isotope exchange according to first-order kinetics indicated that up to 3 years are needed to attain equilibrium. Since, however, metal-humic interaction remains reversible, exchange of metals between humic carriers and mineral surfaces cannot be neglected on the long time scale to be considered in predictive transport models.

1. INTRODUCTION

Migration of multivalent toxic or radiotoxic metals, which are strongly adsorbed to mineral surfaces at moderate pH, is essentially governed by colloid-borne transport processes. Besides inorganic colloids, humic substances have been recognised to be important as carriers. Their complexing properties can dominate the aqueous species distribution of contaminant metals (Dearlove et al., 1991; Choppin, 1992; Kim et al., 1992). Collecting data on metal-humic complexation as a function of geochemical parameters has been a task for decades (e.g., Town and Filella, 2000; Milne et al., 2003), quantified in the form of conditional or semi-conditional stability constants (e.g., according to the charge neutralisation model (Kim and Czerwinski, 1996)) and of intrinsic equilibrium constants based on mechanistic approaches such as the NICA-Donnan model (Kinniburgh et al., 1996) or Tipping's models (Tipping, 2002), using continuous or discrete site affinity distributions, respectively.

For modelling humic-bound transport of metals, their solid-liquid distribution is considered to be the result of a competitive partitioning process between solid surface and humic colloids. Using equilibrium constants for adsorption and complexation implies that a steady local equilibrium exists under flow conditions. A dynamic exchange of free and adsorbed / complexed state is presumed. A number of models have been proposed (Knabner et al., 1996; Lührmann et al., 1998; Warwick et al., 2000; Schuessler et al., 2001; Schmitt et al., 2003; Bryan et al., 2005; Kim and Kim, 2007), part of them taking adsorption of the humic carriers into account (Knabner et al., 1996; Lührmann et al., 1998; Bryan et al., 2005; Kim and Kim, 2007). Most of these models allow for non-equilibrium conditions in case of slow kinetics compared to flow velocity, using rate constants instead of equilibrium constants (see Lippold and Lippmann-Pipke (2009) for an overview). However, it remains a basic prerequisite that

interactions are reversible and dynamic in nature, i.e., association and dissociation are expected to run permanently.

The validity of this assumption is affected by a phenomenon that appears to be characteristic of higher-valent metals: Their ability to dissociate from humic complexants diminishes with increasing time of contact. Such rise in complex inertness (i.e., decrease in the rate of dissociation) was found in batch experiments with competing ligands (Cacheris and Choppin, 1987; Choppin and Clark, 1991) as well as with cation exchange resins (King et al., 2001; Geckeis et al., 2002). Implications on metal transport became manifest in column experiments (Rao et al., 1994; Artinger et al., 1998), showing that retardation is more and more reduced the longer the complex has been pre-equilibrated prior to injection, i.e., interaction of the metal with the stationary phase is increasingly prevented. These inertisation processes were observed for humate or fulvate complexes of Eu(III) (Rao et al., 1994; King et al., 2001; Geckeis et al., 2002), Am(III) (Artinger et al., 1998), Th(IV) (Cacheris and Choppin, 1987) and U(VI) (Choppin and Clark, 1991; Rao et al., 1994), within time frames ranging from 2 days to 6 months.

Most authors supposed some kind of diffusion process where bound metals move from surface sites to deeper sites inside the humic molecule. However, it was found that competition effects of Al(III) and Fe(III), pre-equilibrated with humic acid before introducing a second metal, show an increase as well, i.e., complexation of the subsequently added metal is more and more suppressed with increasing time of pre-equilibration (Lippold et al., 2007; Lippold et al., 2012). Diffusion of the first metal away from the initial sites should, on the contrary, facilitate complexation of the second metal. An on-site inertisation, e.g., in consequence of induced conformational changes, seems more probable, even though spectroscopic evidence of structural rearrangements could not be provided so far.

Irrespectively of the mechanistic background, the question arises as to whether reversibility is impaired in this process. If, in the end, humic-bound metals are to be regarded as "fixed", this

will entail completely different predictions of their migration behaviour than in the case of a dynamic partitioning process. Results from column experiments were successfully described by assuming such transition from an exchangeable state to a non-exchangeable state (KICAM approach and K1D transport code) (Schuessler et al., 2000; Warwick et al., 2000; Schuessler et al., 2001; Bryan et al., 2005).

The objective of this work was to obtain an improved understanding of the equilibrium characteristics of metal-humate complexation, particularly with regard to possible inertisation processes and their consequences in respect of reversibility. For "looking into" the dynamics of an equilibrium, radiotracer experiments are uniquely suited. A radioactive probe, introduced as a reactant into pre-equilibrated systems, will represent the overall equilibrium if there is a dynamic exchange. In case of a static equilibrium, however, the tracer will not get involved because the reaction has stopped, also on a molecular level. Adsorption or binding processes are then a simple "sticking". In a previous paper, the tracer exchange principle was applied to elucidate the dynamics of adsorption equilibria of humic substances (Lippold and Lippmann-Pipke, 2014). Several isotope exchange studies on metal-humate complexation can be found in the literature (Colston et al., 1997; Marx and Heumann, 1999; Mizera et al., 2001), all of which using separation methods like ultrafiltration, dialysis or size exclusion chromatography. Implications of inertisation effects were not considered so far.

The present study was performed with terbium(III) and the radioisotope ^{160}Tb (half-life: 72.3 days), contacted with humic acids for time periods up to 3 months corresponding to the time frame reported for inertisation phenomena. Flocculation was employed as a separation mechanism, avoiding techniques that are potentially invasive. Tb(III) was chosen as a representative of higher-valent metals. It is widely accepted that trivalent lanthanides are, to some extent, suitable as analogues of trivalent actinides such as Am(III), Cm(III) or Pu(III) (Choppin, 1995), which are in the focus of safety analyses for final repositories because of their long half-lives.

2. MATERIALS AND METHODS

2.1. Materials

Non-radioactive chemicals and humic acid (HA) were supplied by Sigma-Aldrich (Germany). Another humic acid was isolated from surface water collected on the raised bog "Kleiner Kranichsee" (near Carlsfeld, Germany). Separation was carried out according to the recommendations of the International Humic Substances Society (Aiken, 1985). Both humic materials were purified by repeated precipitation and redissolution with 0.1 M HCl and 0.1 M NaOH / 0.01 M NaF, respectively, followed by dialysis and lyophilisation. Characteristics of these substances are given elsewhere (Lippold et al., 2005a; Lippold et al., 2005b). $[^{160}Tb]TbCl_3$ (activated up to 1 GBq per mg Tb) was purchased from Radioisotope Centre Polatom (Poland).

2.2. Isotope exchange experiments

Stock solutions of 0.1 M $NaClO_4$ and of humic acid, $Tb(NO_3)_3$ as well as $[^{160}Tb]TbCl_3$ in 0.1 M $NaClO_4$, all adjusted to a pH of 4.0, were dispensed into 5 mL centrifuge tubes at variable amounts, giving a total volume of 4 mL. The component to be added finally after a pre-equilibration step was introduced in a small volume of 20 µL, keeping the concentrations of the other components virtually unchanged. The solutions were rotated end-over-end during equilibration. Changes in pH did not occur.

In a first series of experiments (illustrated in Fig. 1), 0.5 g L^{-1} humic acid was contacted with Tb (natural stable isotope ^{159}Tb) at a range of concentrations (0.3 mM - 2.4 mM), covering a binding isotherm up to the state of saturation. The systems were completed by adding 1 nM of [^{160}Tb]Tb as a tracer, either instantaneously or subsequently after different time periods of pre-equilibration. Owing to the high metal loads, flocculation of humic colloids generates a solid-liquid system where adsorbed amounts of ^{160}Tb can be determined by radiometric analysis of the supernatant after centrifugation (2000 rpm). For the case of full reversibility, it is presumed that ^{160}Tb represents the behaviour of total Tb. Its concentration was determined by means of a gamma counter 1480 Wallac Wizard 3" (Perkin Elmer, USA) from the count rate relative to freshly prepared reference solutions of [^{160}Tb]$TbCl_3$ in 0.1 M $HClO_4$. Flocculation was verified to be nearly complete (99% after centrifugation) based on UV absorption at 254 nm, using a Lambda 25 spectrophotometer (Perkin Elmer, USA).

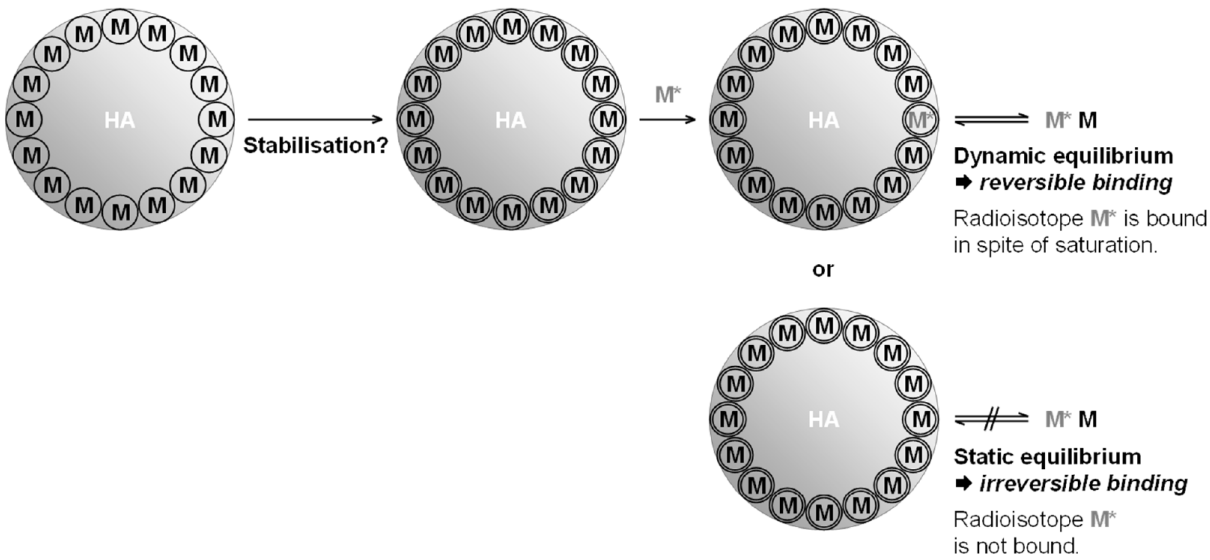

Fig. 1. Isotope exchange experiments for probing the equilibrium characteristics of metal-humate complexation (HA: humic acid, M: metal, M*: radioisotope), starting from the state of saturation.

In a modified procedure (Fig. 2), 1 nM of [^{160}Tb]Tb was first added to 0.5 g L^{-1} humic acid, followed by saturation with non-radioactive ^{159}Tb after pre-equilibration for different periods of time. A fixed concentration within the plateau region of the binding isotherm (2 mM ^{159}Tb) was chosen in these experiments, and data were quantified referring to [^{160}Tb]Tb instead of total Tb. Wall adsorption of [^{160}Tb]Tb during pre-equilibration was ascertained to be insignificant by checking the concentration in parallel tests. Humic-bound amounts of [^{160}Tb]Tb in the non-saturated system (where no flocculation occurs) were determined by ultrafiltration, using Vivaspin 2 kDa centrifuge filters with Hydrosart® membrane (Sartorius, Germany). Retention was 97% for humic acid and 4% for aqueous Tb at the experimental conditions.

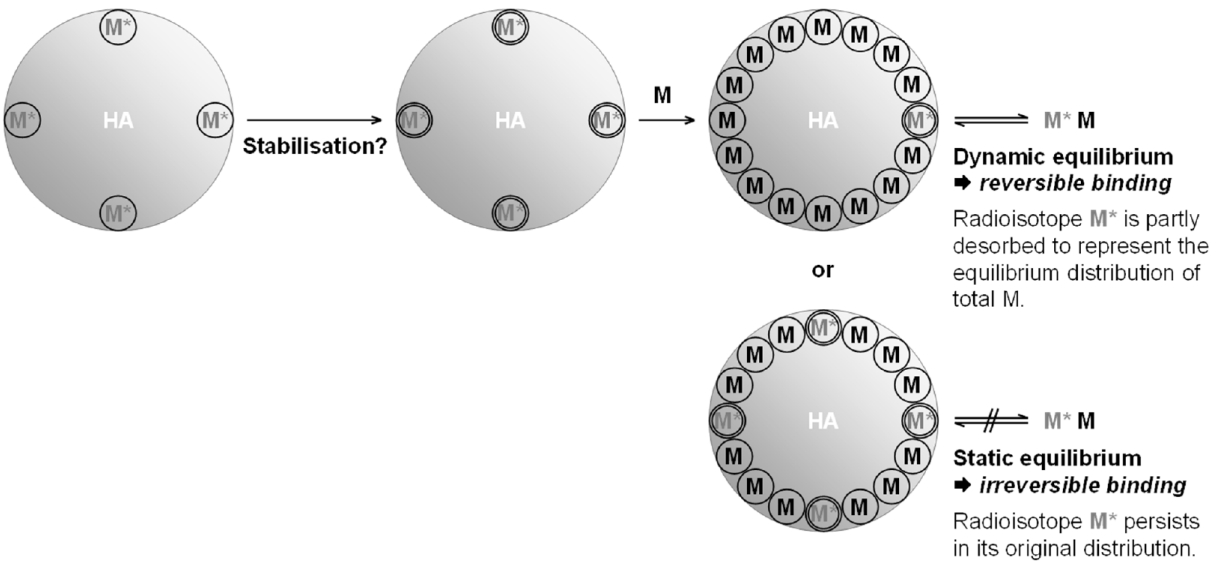

Fig. 2. Isotope exchange experiments in a reverse procedure, starting from a very low metal load with the radioisotope M*.

3. RESULTS

Figure 3 shows isotherms for binding of Tb to humic acid, obtained with ^{160}Tb as a radiotracer. The square symbols show the results of conventional experiments where the tracer was simultaneously added together with the bulk of non-radioactive Tb. The interaction parameters given in the caption are based on the charge neutralisation model (Kim and Czerwinski, 1996), which corresponds to the Langmuir equation with an equilibrium constant normalised to ion charge (stability constant β) and a maximum adsorption normalised to proton exchange capacity and ion charge (loading capacity LC). At equilibrium concentrations higher than 1 mM, a plateau level is reached, indicating that more binding sites are not available under the given conditions. In a static equilibrium, where binding is simply adhesion without any dynamic exchange, all sites will be blocked in this state of saturation.

However, if the radiotracer is subsequently introduced after Tb and humic acid have already been pre-equilibrated (triangle symbols), coincident isotherms are obtained. The radioactive probe represents the solid-liquid distribution of total Tb, even though it encounters the state of maximum occupancy. It is thus evident that there is a permanent exchange between dissolved and humic-bound Tb; the tracer is involved in a dynamic equilibrium.

In additional tests, complete equilibration was also observed when reducing the time of contact with the radiotracer to 15 minutes instead of 1 day (Supplementary Material, Fig. A.1). This indication of a rapid isotope exchange is consistent with a spectroscopic study on Cm(III)-humate complexation (Freyer et al., 2009). From the profile of fluorescence decay in D_2O at variable excitation wavelengths, it was concluded that there is a fast interchange between aquo ion and humate complex on the time scale of the fluorescence lifetime (less than a millisecond).

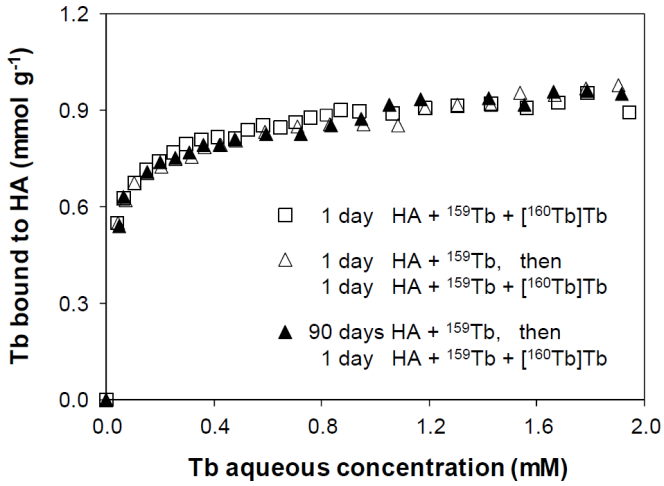

Fig. 3. Isotherms of binding of Tb(III) to humic acid, obtained with ^{160}Tb(III) as a radiotracer, which was added simultaneously or subsequently after different times of pre-equilibration with non-radioactive ^{159}Tb(III) (1 nM [^{160}Tb]Tb, 0.5 g L^{-1} HA Aldrich, 0.1 M $NaClO_4$, pH 4.0). Interaction parameters: log (β / L mol^{-1}) = 5.02, LC = 49.7% referring to a proton exchange capacity of 5.43 meq g^{-1} (Kim et al., 1990).

Notably, there is no indication of an inertisation process, even after extended periods of pre-equilibration (90 days). Most reported observations refer to a similar or a shorter time frame. Metal loads were, however, far below saturation in these studies. An influence of concentration is conceivable since humic molecules comprise a multitude of binding sites with a broad affinity distribution (Tipping, 2002). A small fraction of irreversibly bound Tb could be "hidden" behind the isotherm.

For this reason, the experimental approach was modified by reversing the sequence of introducing the isotopes: At first, humic acid was contacted with [^{160}Tb]Tb at a very low concentration. Subsequently, the large excess of ^{159}Tb was added at a concentration in the range of saturation. For representing the solid-liquid distribution of total Tb in a dynamic equilibrium, the radioisotope was expected to be partly desorbed since the bound fraction of total Tb is lower in the plateau region of the binding isotherm.

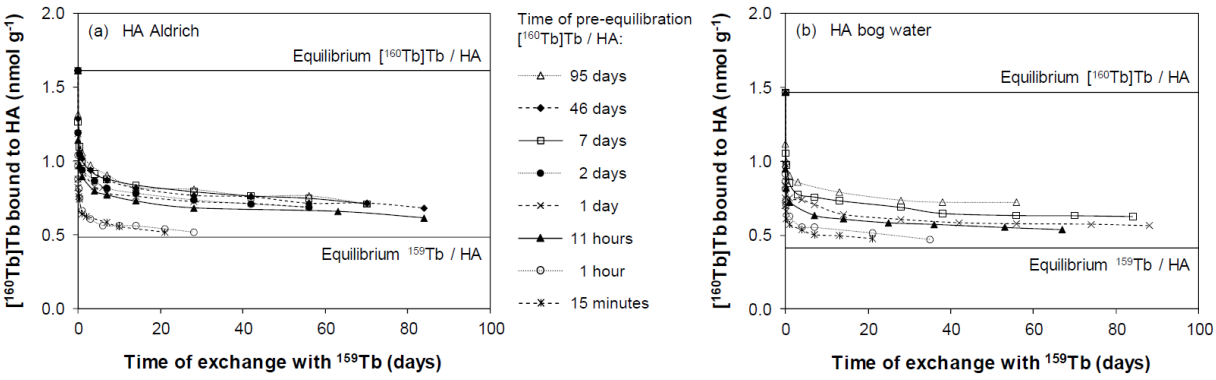

Fig. 4. Amount of [^{160}Tb]Tb(III) bound to humic acids after saturating with non-radioactive ^{159}Tb(III), shown as a function of exchange time for different times of pre-equilibration (1 nM [^{160}Tb]Tb, 2 mM ^{159}Tb, 0.5 g L^{-1} HA, 0.1 M $NaClO_4$, pH 4.0).

Figure 4 shows the results of these experiments, conducted with two different humic materials. The upper equilibrium lines show their initial loads with [^{160}Tb]Tb prior to adding ^{159}Tb. The lower equilibrium lines show the amounts of bound [^{160}Tb]Tb representing the bound fraction of total Tb (mostly ^{159}Tb) in the case of simultaneous addition. If the non-radioactive isotope is introduced later, desorption of the radiotracer is in fact initiated, striving towards the equilibrium state ^{159}Tb / HA – however, at much lower rates compared to the equilibration process in the reverse procedure.

Moreover, the rates proved to be dependent on the time of pre-equilibration with [^{160}Tb]Tb. Equilibrium is closely approached after 1 month of exchange for the shortest pre-equilibration times, whereas bound amounts are still far away from equilibrium after 3 months if more time is admitted for pre-equilibration. Obviously, desorption of [^{160}Tb]Tb is increasingly hindered. The existence of kinetic inertisation phenomena was thus substantiated. However, it appears that they are confined to the most reactive sites, which are sought by the very small fraction of radiolabelled Tb. (Evidence for the successive occupation of strong and weak sites with increasing metal concentration was presented, e.g., by Rabung and Geckeis (2009) and by Kautenburger et al. (2014).)

Remarkably, results for HA Aldrich and HA bog water are comparable. The coal-derived Aldrich material is subject to some criticism because its provenance is not very close to environmental systems. Nonetheless, its behaviour seems to be in line with "real" natural humic acids, as was also found in many other studies (e.g., Kim et al., 1990; Reiller et al., 2002; Hur and Schlautman, 2003).

The time-dependent progress of inertisation can be seen in Fig. 5, where adsorbed amounts after a fixed time of exchange (1 h) are plotted as a function of pre-equilibration time. Changes are most pronounced in the first days, followed by a very minor trend over an extended time period. A comparable time dependence was found in most related studies (Cacheris and Choppin, 1987; Choppin and Clark, 1991; Rao et al., 1994; Lippold et al., 2012).

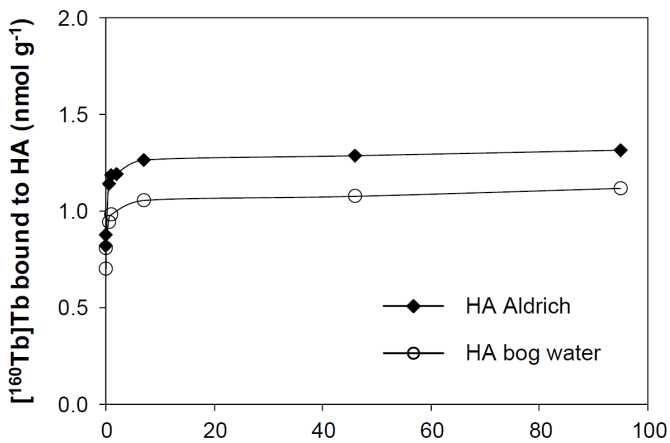

Fig. 5. Amount of [^{160}Tb]Tb(III) bound to humic acids after 1 h of exchange with ^{159}Tb(III), shown as a function of pre-equilibration time (conditions as in Fig. 4).

4. DISCUSSION

4.1. Possible mechanisms behind the inertisation process

Attempts were made to elucidate the chemical background of the present findings by means of time-resolved laser-induced fluorescence spectroscopy, using Eu(III) as a probe, which is very sensitive towards structural alterations in its surroundings. Significant spectral changes within the time frame of the strongest progress of inertisation could not be detected, possibly because a higher metal concentration (by two orders of magnitude compared to the radiotracer) was necessary to obtain sufficient fluorescence intensity. The weaker sites, occupied at this concentration, are perhaps not concerned by the kinetic effect. (Spectra and instrumental details are provided in the Supplementary Material, Fig. A.2.)

In a recent paper (Sherriff et al., 2015), a comparable kinetic stabilisation was reported for desorption of Eu(III) from bentonite, interestingly on the same time scale as observed in this study. The process was explained by slow diffusion into interlayer spaces since structural changes can be ruled out for clay minerals.

Inertisation of metal-humic complexes was often interpreted in a similar way. Many authors arrived at the conclusion that metal ions penetrate humic colloids, changing from labile peripheral positions to internal positions where they get entangled and dissociation is more complicated (Cacheris and Choppin, 1987; Choppin and Clark, 1991; Rao et al., 1994; Engebretson and von Wandruszka, 1998; King et al., 2001). Just the fact, however, that kinetic effects are only observed at very low concentrations where the strongest sites are occupied is an argument in favour of an on-site process rather than in-diffusion. Residues of naturally inherent metals in the humic materials (Lippold et al., 2005b) could be desorbed over time, resulting in structural rearrangements within the humic network that impede isotope exchange. In the absence of spectroscopic evidence of a changing microenvironment,

an alternative approach to an explanation arises from substantial contents of Fe (3.3 mg g^{-1} for HA Aldrich, 22.4 mg g^{-1} for HA bog water (Lippold et al., 2005b)), probably in the form of small particles that may adsorb part of Tb. These Fe clusters could undergo alterations in contact with water (dissolution / remineralisation). Since, however, results are not influenced by the standing time of the HA stock solutions before the experiments, it is more probable that structural changes are induced by the reacting metal itself. Intra- or intermolecular bridging effects are thinkable. Recent concepts describe dissolved humic substances as loose associations of relatively small entities, often compared to micelles (see Sutton and Sposito (2005) for a review). It was found that extensive purification from metals leads to disaggregation (Simpson et al., 2002).

Bacterial degradation may be an issue in HA solutions that are used for a longer time. Sterilisation measures were not taken. Nonetheless, microbial activity is not likely to be causative of the phenomenon because the inertisation process declines after several days (Fig. 5). The coincident course of the binding isotherms of Tb determined at a temporal distance of 90 days (Fig. 3) can be taken as an indication that the complexing groups are not significantly altered by microbes within the experimental time frame.

4.2. Kinetic analysis of isotope exchange

For short pre-equilibration times, the kinetics of exchange (Fig. 4) is superimposed by the kinetics of inertisation (Fig. 5), resulting in a prolongation of the process. This interference is much less pronounced for longer times of pre-equilibration, and a kinetic analysis of the exchange process itself is possible, neglecting the remaining minor progress of inertisation. It can be shown that isotope exchange always follows first-order kinetics, regardless of the

mechanism of the exchange reaction (Duffield and Calvin, 1946). For the system under study, the rate of approach to equilibrium is given by Eq. (1)

$$-\frac{\mathrm{d}c_{\mathrm{ads}}}{\mathrm{d}t} = k\left(c_{\mathrm{ads}} - c_{\mathrm{ads}}^{\mathrm{eq}}\right), \tag{1}$$

where k is the rate constant, c_{ads} is the concentration of humic-bound [^{160}Tb]Tb at time t after saturation, and $c_{\mathrm{ads}}^{\mathrm{eq}}$ is its concentration at equilibrium, i.e., corresponding to the distribution of total Tb (designated by ^{159}Tb / HA in Fig. 4). Integration yields Eq. (2) for the time course of isotope exchange

$$c_{\mathrm{ads}} = \left(c_{\mathrm{ads}}^{\mathrm{ini}} - c_{\mathrm{ads}}^{\mathrm{eq}}\right) e^{-kt} + c_{\mathrm{ads}}^{\mathrm{eq}}, \tag{2}$$

where $c_{\mathrm{ads}}^{\mathrm{ini}}$ is the initial concentration of humic-bound [^{160}Tb]Tb. Fitting this monoexponential equation to the data was not successful, due to the very steep decrease for short times of exchange. In a modified approach, the total amount of sites that are initially occupied by [^{160}Tb]Tb was split into a fast exchanging and a slowly exchanging component, characterised by the rate constants k_1 and k_2, respectively. Separate integration and addition (considering that both types of sites are equally occupied at the initial state as well as at the equilibrium state) results in Eq. (3)

$$c_{\mathrm{ads}} = x\left(c_{\mathrm{ads}}^{\mathrm{ini}} - c_{\mathrm{ads}}^{\mathrm{eq}}\right) e^{-k_1 t} + \left(1-x\right)\left(c_{\mathrm{ads}}^{\mathrm{ini}} - c_{\mathrm{ads}}^{\mathrm{eq}}\right) e^{-k_2 t} + c_{\mathrm{ads}}^{\mathrm{eq}} \tag{3}$$

with x denoting the fraction of fast exchanging sites. Using this equation, the progress of exchange can be well described, as shown for an exemplary data series in Fig. 6. Obviously, the very small fraction of sites occupied by [^{160}Tb]Tb (~ 1/10^6) is still heterogeneous with

respect to exchange kinetics. The actual degree of heterogeneity certainly exceeds two types of sites since their properties are continuously distributed. Fit parameters for the data series obtained for the longest pre-equilibration times are listed in Table 1, together with estimated equilibration times based on extrapolation. According to this, up to 3 years are needed for a stabilised complex to reach the equilibrium state. Both rate constants decrease with increasing time of pre-equilibration, while there is only little variation in the distribution of fast and slowly exchanging sites for both humic materials. This again supports the hypothesis of an on-site inertisation. On the other hand, the decreasing *k* values underline that Eq. (3) is a simplification. (The fits are shown in the Supplementary Material, Fig. A.3.)

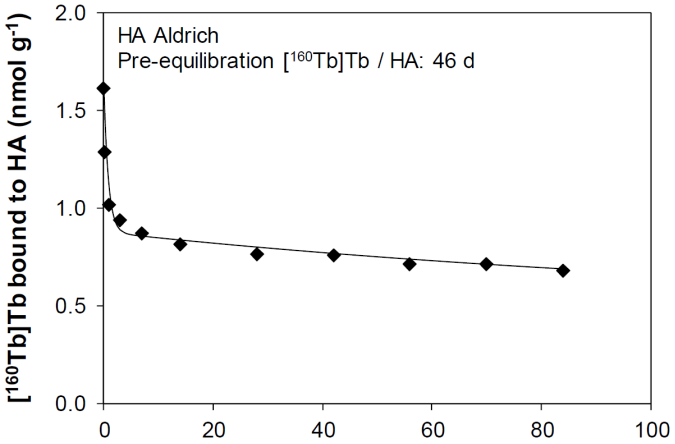

Fig. 6. Kinetic analysis of isotope exchange by fitting Eq. (3) to an exemplary data series (symbols: experimental data, line: fitted curve calculated with the parameters given in Table 1).

Deprotonation at higher pH will promote the participation of less acidic (i.e., stronger) groups, and rate constants will decrease. However, there are no basic changes in the nature of the complexes (Rabung and Geckeis, 2009). Thus, it is improbable that a higher pH would entail deviating results with regard to the dynamic character of the equilibrium, the occurrence of inertisation effects and the influence of metal loads.

Table 1. Fit parameters for the kinetics of isotope exchange according to Eq. (3).

Humic acid	Time of pre-equilibration [^{160}Tb]Tb / HA (d)	k_1 (d^{-1})	k_2 (10^{-3} d^{-1})	x	MSE [a] ($nmol^2$ g^{-2})	$t\,(c_{ads} = c_{ads}^{eq} + 0.01\ c_{ads}^{eq})$ [b] (d)
HA Aldrich	7	2.0	8.1	0.64	0.0101	548
	46	1.2	7.7	0.65	0.0086	573
	95	1.0	7.3	0.65	0.0086	604
HA bog water	7	1.7	6.7	0.66	0.0168	666
	95	1.4	4.0	0.64	0.0198	1129

[a] Mean square error.

[b] Time needed for approaching the equilibrium concentration at a distance of 1%.

4.3. Possible implications of flocculation

The experimental approach applied here differs from desorption studies in the fact that flocculation of humic acid is induced (which does not correspond to the situation in natural systems). Complete occupation of all available sites is necessary for probing a dynamic exchange by subsequent introduction of the radiotracer, otherwise it would be bound to the remaining free sites. In the reverse procedure, the non-radioactive isotope must be added at amounts at least close to saturation since the bound fraction of metal in total should decrease distinctly. Flocculation could be avoided by choosing a very low concentration of humic acid, but this would necessitate separation techniques that are more error-prone.

For flocculated humic acid, a certain hindrance of metal desorption may be expected. Dissociation rates of metal-humic complexes decrease on lowering the pH due to contraction and agglomeration of humic molecules (Rao et al., 1994; Monsallier et al., 2003), which is a pre-stage of flocculation where the colloidal system is no longer stable. High metal loads have the same effect. Augmented aggregation was also found at increased concentrations of humic

acid (Simpson, 2002), as applied in this study. In fact, the rate constants k_2 for slow exchange (Table 1) are lower by one order of magnitude compared to desorption rate constants reported by King et al. (2001) and Geckeis et al. (2002) for slowly desorbing fractions of Eu(III) in dilute solutions of humic or fulvic acid.

Regarding the first procedure starting from saturation, flocculation could be an alternative explanation for the absence of an ageing effect at high metal loads. Structural rearrangements, as a possible mechanism of the inertisation process, might be impeded or prevented. However, it seems more probable that such rearrangements like intra- or intermolecular bridging are rather facilitated if the repulsive forces within the humic structure are reduced by charge compensation (i.e., the cause of flocculation).

5. CONCLUSIONS

A direct analysis of the equilibrium characteristics of metal-humate complexation was performed by using the isotope exchange principle. Evidence was provided that this interaction is a dynamic equilibrium process. Special attention was paid to consequences of possible ageing effects. It was found that the metal / humic concentration ratio plays an essential role for the occurrence of inertisation phenomena.

Full reversibility is a prerequisite for the applicability of thermodynamic constants in speciation and transport modelling, but exchange rates decrease considerably as (initial) metal loads are lowered, and inertisation processes come into effect. A humic complex, loaded with trace amounts of higher-valent metals and "aged" for some time, will not be in equilibrium with dissolved reactants or mineral surfaces if conditions change relatively fast, e.g., in case of rapid movement. This is not necessarily applicable to divalent trace metals, where interaction with humic complexants is less intense. The long-term behaviour of these metals is

still in need of investigation. At pH values more away from the acidic edge of environmental conditions, exchange rates will be different, but the basic conclusions regarding reversibility and inertisation in dependence on metal loads certainly remain unaffected. The finding that only the stronger sites are concerned by ageing effects contradicts the prevailing notion of an in-diffusion as underlying mechanism.

To decide how to treat higher-valent humic-bound metals in modelling, two aspects of the present results are important: (I) The inertisation process declines after a fairly short time period (Fig. 5). A steady state is not yet attained after 95 days, but it appears unlikely that the remaining minor trend continues endlessly. (II) The ageing effect does not lead to irreversible binding (Fig. 4). For longer contact times, complete equilibration could not be observed within the experimental time frame, but the results for short contact times give rise to the assumption that the equilibrium state will be reached if sufficient time is admitted, which is also supported by the extrapolative kinetic data analysis. Whether humic-bound metals take part in interaction processes is thus a question of the time scale on which conditions or positions are changing. Equilibration times of several years are still very short compared to the time frame to be covered in safety assessments for nuclear waste repositories. Nearly stagnant systems are to be considered, where steady equilibria may exist in spite of very slow kinetics. Assuming a trend towards a non-exchangeable state, as is done in current models, is certainly a suitable approach on the time scale of column experiments, but probably not adequate if time periods available for equilibration are extremely long. Under these conditions, metal exchange between humic carriers and mineral surfaces cannot be neglected in predictive transport calculations, and equilibrium constants are fully applicable. Uncertainties of course remain as long as the mechanism of inertisation is not completely understood. X-ray spectroscopic methods such as EXAFS could shed more light on temporal changes in the chemical environment of humic-bound metals.

ACKNOWLEDGEMENTS

This work was funded by the German Federal Ministry for Economic Affairs and Energy (BMWi), support code 02 E 10971. The authors would like to thank Prof. Dr. H. Geckeis (Karlsruhe Institute of Technology) for valuable advice.

APPENDIX A. SUPPLEMENTARY DATA

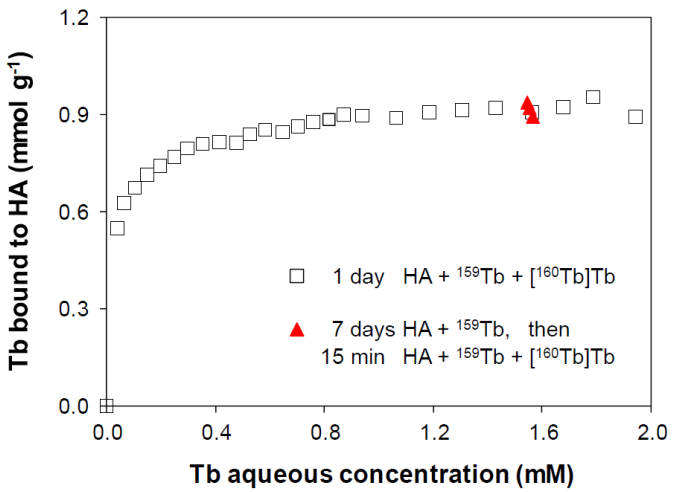

Fig. A.1. Isotope exchange experiments according to the procedure applied in Fig. 3, admitting a shorter time for exchange (1 nM [^{160}Tb]Tb, 2 mM ^{159}Tb, 0.5 g L^{-1} HA Aldrich, 0.1 M $NaClO_4$, pH 4.0).

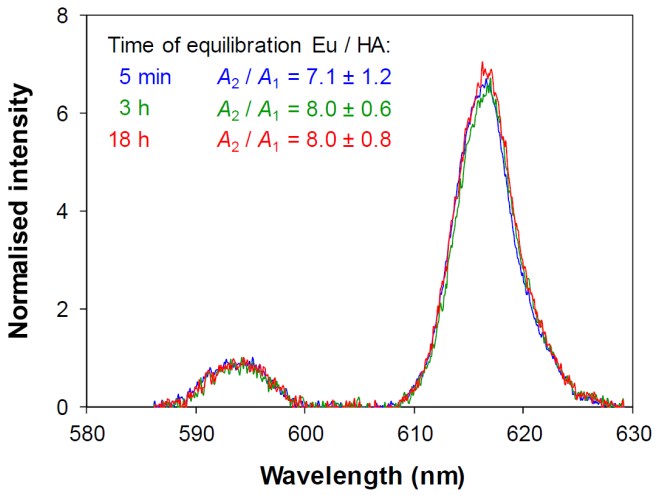

Fig. A.2. Fluorescence spectra of Eu(III) after different times of contact with humic acid (100 nM Eu, 0.5 g L^{-1} HA Aldrich, 0.1 M $NaClO_4$, pH 4.0), measured by means of time-resolved laser-induced fluorescence spectroscopy (TRLFS) in a time window between 10 µs and 2 ms after laser flash, skipping the fast-decaying fluorescence of the humic acid. Accumulated intensities were baseline-corrected and normalised to the maximum of emission band (1) at the wavelength of 595 nm. Ratios of integral intensities (band areas *A*) are based on Gaussian fits. At the chosen excitation wavelength of 356 nm, Eu is indirectly excited by radiationless energy transfer from the humic ligands. A pulsed Nd:YAG laser (Spectra-Physics Quanta-Ray, USA), operating at 20 Hz, was used in combination with an optical parametric oscillator (GWU Lasertechnik, Germany). The fluorescence emission was recorded by an ICCD camera (iStar DH720, Andor Technology, UK) coupled to a spectrograph (MS257, Oriel Instruments, USA).

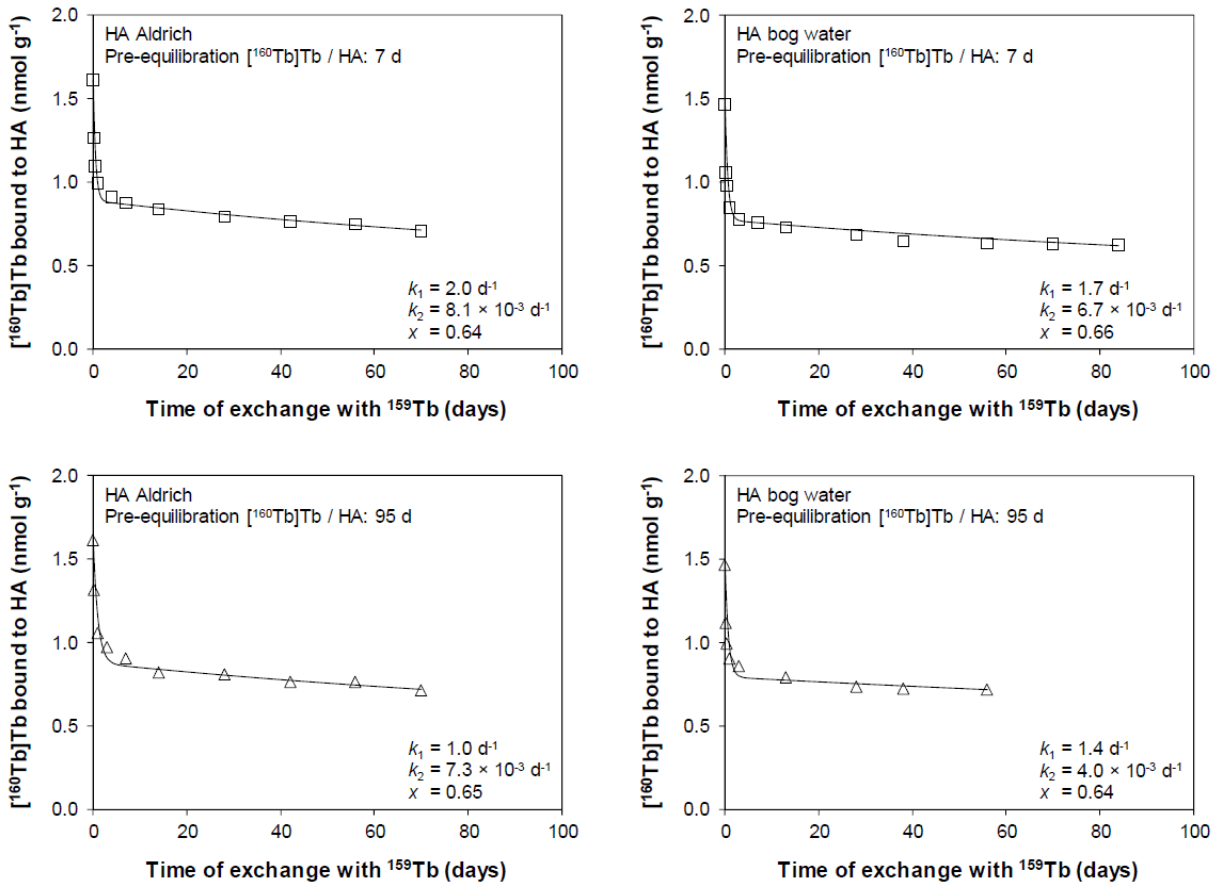

Fig. A.3. Fits according to Eq. (3) for additional data series from Fig. 4.

REFERENCES

Aiken G. R. 1985 Isolation and concentration techniques for aquatic humic substances. In *Humic substances in soil, sediment and water: Geochemistry and isolation* (eds. G. R. Aiken, D. M. MacKnight, R. L. Wershaw and P. MacCarthy). Wiley-Interscience, New York, pp. 363–385.

Artinger R., Kienzler B., Schüßler W. and Kim J.-I. 1998 Effects of humic substances on the ^{241}Am migration in a sandy aquifer: column experiments with Gorleben groundwater/sediment systems. *J. Contam. Hydrol.* **35**, 261–275.

Bryan N. D., Barlow J., Warwick P., Stephens S., Higgo J. J. and Griffin D. 2005 The simultaneous modelling of metal ion and humic substance transport in column experiments. *J. Environ. Monitor.* **7**, 196–202.

Cacheris W. P. and Choppin G. R. 1987 Dissociation kinetics of thorium-humate complex. *Radiochim. Acta* **42**, 185–190.

Choppin G. R. 1992 The role of natural organics in radionuclide migration in natural aquifer systems. *Radiochim. Acta* **58/59**, 113–120.

Choppin G. R. 1995 Comparative solution chemistry of the 4f and 5f elements. *J. Alloy Cpd.* **223**, 174–179.

Choppin G. R. and Clark S. B. 1991 The kinetic interactions of metal ions with humic acids. *Mar. Chem.* **36**, 27–38.

Colston B. J., van Elteren J. T., Kolar Z., I. and de Goeij J. J. M. 1997 Kinetics in a Eu(III)-humic acid system by isotopic exchange with $^{152m}Eu^{3+}$ and size exclusion chromatography - a feasibility study. *Radiochim. Acta* **78**, 111–115.

Dearlove J. P. L., Longworth G., Ivanovich M., Kim J.-I., Delakowitz B. and Zeh P. 1991 A study of groundwater colloids and their geochemical interactions with natural radionuclides in Gorleben aquifer systems. *Radiochim. Acta* **52/53**, 83–89.

Duffield R. B. and Calvin M. 1946 The stability of chelate compounds. III. Exchange reactions of copper chelate compounds. *J. Am. Chem. Soc.* **68**, 557–561.

Engebretson R. R. and von Wandruszka R. 1998 Kinetic aspects of cation-enhanced aggregation in aqueous humic acids. *Environ. Sci. Technol.* **32**, 488–493.

Freyer M., Walther C., Stumpf T., Buckau G. and Fanghänel T. 2009 Formation of Cm humate complexes in aqueous solution at pH_c 3 to 5.5: The role of fast interchange. *Radiochim. Acta* **97**, 547–558.

Geckeis H., Rabung T., Ngo Manh T., Kim J.-I. and Beck H. P. 2002 Humic colloid-borne natural polyvalent metal ions: dissociation experiment. *Environ. Sci. Technol.* **36**, 2946–2952.

Hur J. and Schlautman M. A. 2003 Molecular weight fractionation of humic substances by adsorption onto minerals. *J. Colloid Interf. Sci.* **264**, 313–321.

Kautenburger R., Hein C., Sander J. M. and Beck H. P. 2014 Influence of metal loading and humic acid functional groups on the complexation behavior of trivalent lanthanides analyzed by CE-ICP-MS. *Anal. Chim. Acta* **816**, 50–59.

Kim J.-I., Buckau G., Li G. H., Duschner H. and Psarros N. 1990 Characterization of humic and fulvic acids from Gorleben groundwater. *Fresen. J. Anal. Chem.* **338**, 245–252.

Kim J.-I. and Czerwinski K. R. 1996 Complexation of metal ions with humic acid: Metal ion charge neutralization model. *Radiochim. Acta* **73**, 5–10.

Kim J.-I., Zeh P. and Delakowitz B. 1992 Chemical interactions of actinide ions with groundwater colloids in Gorleben aquifer systems. *Radiochim. Acta* **58/59**, 147–154.

Kim M. and Kim S. B. 2007 Modeling contaminant transport in a three-phase groundwater system with the Freundlich-type retardation factor. *Environ. Technol.* **28**, 205–216.

King S. J., Warwick P., Hall A. and Bryan N. D. 2001 The dissociation kinetics of dissolved metal–humate complexes. *Phys. Chem. Chem. Phys.* **3**, 2080–2085.

Kinniburgh D. G., Milne C. J., Benedetti M. F., Pinheiro J. P., Filius J., Koopal L. K. and Van Riemsdijk W. H. 1996 Metal ion binding by humic acid: Application of the NICA-Donnan model. *Environ. Sci. Technol.* **30**, 1687–1698.

Knabner P., Totsche K. U. and Kögel-Knabner I. 1996 The modeling of reactive solute transport with sorption to mobile and immobile sorbents: 1. Experimental evidence and model development. *Water Resour. Res.* **32**, 1611–1622.

Lippold H., Eidner S., Kumke M. U. and Lippmann-Pipke J. 2012 Diffusion, degradation or on-site stabilisation – Identifying causes of kinetic processes involved in metal-humate complexation. *Appl. Geochem.* **27**, 250–256.

Lippold H., Evans N. D. M., Warwick P. and Kupsch H. 2007 Competitive effect of iron(III) on metal complexation by humic substances: Characterisation of ageing processes. *Chemosphere* **67**, 1050–1056.

Lippold H. and Lippmann-Pipke J. 2009 Effect of humic matter on metal adsorption onto clay materials: Testing the linear additive model. *J. Contam. Hydrol.* **109**, 40–48.

Lippold H. and Lippmann-Pipke J. 2014 New insights into the dynamics of adsorption equilibria of humic matter as revealed by radiotracer studies. *Geochim. Cosmochim. Acta* **133**, 362–371.

Lippold H., Mansel A. and Kupsch H. 2005a Influence of trivalent electrolytes on the humic colloid-borne transport of contaminant metals: competition and flocculation effects. *J. Contam. Hydrol.* **76**, 337–352.

Lippold H., Müller N. and Kupsch H. 2005b Effect of humic acid on the pH-dependent adsorption of terbium (III) onto geological materials. *Appl. Geochem.* **20**, 1209–1217.

Lührmann L., Noseck U. and Tix C. 1998 Model of contaminant transport in porous media in the presence of colloids applied to actinide migration in column experiments. *Water Resour. Res.* **34**, 421–426.

Marx G. and Heumann K. G. 1999 Mass spectrometric investigations of the kinetic stability of chromium and copper complexes with humic substances by isotope-labelling experiments. *Fresen. J. Anal. Chem.* **364**, 489–494.

Milne C. J., Kinniburgh D. G., van Riemsdijk W. H. and Tipping E. 2003 Generic NICA−donnan model parameters for metal-ion binding by humic substances. *Environ. Sci. Technol.* **37**, 958–971.

Mizera J., Beneš P., Hvoždová I. and Jansová A. 2001 Application of radiotracer techniques to a kinetic study of the interaction of Eu with humic acid. *Radiochim. Acta* **89**, 785–789.

Monsallier J.-M., Schuessler W., Buckau G., Rabung T., Kim J.-I., Jones D., Keepax R. and Bryan N. 2003 Kinetic investigation of Eu(III)−humate interactions by ion exchange resins. *Anal. Chem.* **75**, 3168–3174.

Rabung T. and Geckeis H. 2009 Influence of pH and metal ion loading on the Cm(III) humate complexation: a time resolved laser fluorescence spectroscopy study. *Radiochim. Acta* **97**, 265–271.

Rao L., Choppin G. R. and Clark S. B. 1994 A study of metal-humate interactions using cation exchange. *Radiochim. Acta* **66**, 141–147.

Reiller P., Moulin V., Casanova F. and Dautel C. 2002 Retention behaviour of humic substances onto mineral surfaces and consequences upon thorium(IV) mobility: Case of iron oxides. *Appl. Geochem.* **17**, 1551–1562.

Schmitt D., Saravia F., Frimmel F. H. and Schuessler W. 2003 NOM-facilitated transport of metal ions in aquifers: importance of complex-dissociation kinetics and colloid formation. *Water Res.* **37**, 3541–3550.

Schuessler W., Artinger R., Kienzler B. and Kim J.-I. 2000 Conceptual modeling of the humic colloid-borne americium(III) migration by a kinetic approach. *Environ. Sci. Technol.* **34**, 2608–2611.

Schuessler W., Artinger R., Kim J.-I., Bryan N. D. and Griffin D. 2001 Numerical modeling of humic colloid borne Americium (III) migration in column experiments using the transport/speciation code K1D and the KICAM model. *J. Contam. Hydrol.* **47**, 311–322.

Sherriff N., Issa R., Morris K., Livens F., Heath S. and Bryan N. D. 2015 Reversibility in radionuclide/bentonite bulk and colloidal ternary systems. *Mineral. Mag.* **79**, 1307–1315.

Simpson A. J. 2002 Determining the molecular weight, aggregation, structures and interactions of natural organic matter using diffusion ordered spectroscopy. *Magn. Reson. Chem.* **40**, S72–S82.

Simpson A. J., Kingery W. L., Hayes M. H. B., Spraul M., Humpfer E., Dvortsak P., Kerssebaum R., Godejohann M. and Hofmann M. 2002 Molecular structures and associations of humic substances in the terrestrial environment. *Naturwissenschaften* **89**, 84–88.

Sutton R. and Sposito G. 2005 Molecular Structure in Soil Humic Substances: The New View. *Environ. Sci. Technol.* **39**, 9009–9015.

Tipping E. 2002 *Cation binding by humic substances*. Cambridge University Press, Cambridge.

Town M. R. and Filella M. 2000 A comprehensive systematic compilation of complexation parameters reported for trace metals in natural waters. *Aquat. Sci.* **62**, 252–295.

Warwick P. W., Hall A., Pashley V., Bryan N. D. and Griffin D. 2000 Modelling the effect of humic substances on the transport of europium through porous media: a comparison of equilibrium and equilibrium/kinetic models. *J. Contam. Hydrol.* **42**, 19–34.