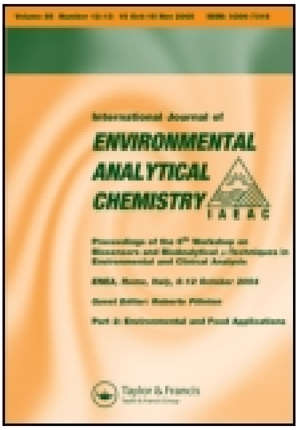

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/geac20

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To cite this article: Danielle Goveia, Fabiana A. Lobo, Iramaia C. Bellin, Leonardo F. Fraceto, Amauri A. Menegário, Peter Burba & André H. Rosa (2011): In situ differentiation of labile/inert metal species in Brazilian tropical rivers by means of a time-controlled batch-procedure based on TEPHA resin, International Journal of Environmental Analytical Chemistry, 91:13, 1296-1309

To link to this article: http://dx.doi.org/10.1080/03067319.2010.484883

Intern. J. Environ. Anal. Chem.
Vol. 91, No. 13, November 2011, 1296–1309

In situ differentiation of labile/inert metal species in Brazilian tropical rivers by means of a time-controlled batch-procedure based on TEPHA resin

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(*Received 18 May 2009; final version received 6 April 2010*)

The present study deals with a new analytical procedure based on a cellulose diffusion membrane and immobilised tetraethylene-pentamine-hexaacetate chelator (DM-TEPHA) for an *in situ* differentiation of labile and inert metal species in aquatic systems. The DM-TEPHA system was prepared by placing TEPHA chelator in pre-purified cellulose bags and *in situ* applied immersing the system in two Brazilian rivers to study the relative lability of metal species (Cu, Pb, Fe, Mn and Ni) as a function of the time and the quantity of exchanger, respectively. The procedure is simple and enables a new perspective for understanding the complexation, transport, stability and lability of metal species in aquatic systems rich in organic matter.

Keywords: water; humic substances; lability, metals; aquatic organic matter

1. Introduction

The surge of industrial activities has intensified various environmental problems as seen for example in the deterioration of several ecosystems due to the accumulation of dangerous contaminants such as metals. According to Hart [1], metal ions can be present in aquatic systems in different physicochemical forms and the (bio-)availability of metals is influenced principally by the form found in nature and not only by the total concentration, as believed in the past [2,3].

Templeton [4] defines the term speciation as a description of the abundance and of the different forms of an element in a given sample (species distribution) and the speciation analysis as the analytical activities of identification and/or quantitative measurement of one or more chemical species in this sample. One of the major problems in speciation analysis is the development of analytical procedures that do not disturb the chemical equilibrium of the original forms existing in the matrix of a sample [5]. Hence, there is a disparity between the enormous complexity of a natural system and the simplicity of an experimental model proposed under laboratory-controlled conditions. Aquatic systems

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ISSN 0306–7319 print/ISSN 1029–0397 online

http://dx.doi.org/10.1080/03067319.2010.484883
http://www.tandfonline.com

have different species in distinct chemical quantities and forms, as well as a great variety of organic compounds in different concentrations, which strongly influence the inherent processes of aquatic chemistry [6].

In the last two decades, alternatives have been sought to overcome some of these difficulties and can be found in literature. Particularly, passive sampling methods have shown much promise as tools for measuring aqueous concentrations of a wide range of priority pollutants. Passive samplers usually combine sampling, selective analyte isolation, pre-concentration and, in some cases, species preservation in one step [7]. In a critical review, Tercier-Waeber and Taillefert [8] illustrated that the challenges associated with metal speciation studies, and the progress made with state of the art voltammetric techniques to measure the speciation of metals *in situ*. Separation techniques based on ion exchange (IE) and complexing resins (CR), and micro separation methods such as the Donnan membrane technique (DMT), diffusive gradients in thin-film gels (DGT) and the permeation liquid membrane (PLM), are among the non-electrochemical methods largely used in this field [9,10]. DGT technique utilises the flux of metal ions through a porous gel matrix into an underlying layer of complexing cation exchange beads (Chelex 100) to quantify the concentration of labile metals. This method has been proposed and widely applied [11,12].

Basically, three kinds of analytical procedures have been used to monitor water quality and to characterise chemical phenomena occurring in water bodies, including metal speciation [6]. Most of these procedures involve sampling, storage, treatment and laboratory analysis/study. These procedures are costly and time-consuming, and can alter the characteristics of the sample, leading to the formation of artefacts. An alternative procedure, called *on site* procedure, consists of measurements/experiments carried out close to the source, i.e. on the banks of the water body and immediately after manual or automatic sampling [13,14]. This procedure is based on procedures and instruments pre-tested in the laboratory and adapted to field conditions, and is ideal for measurements in real time, minimising the production of artefacts that could be generated during the sample's storage. A third kind of procedures, which are still under development and have therefore hardly been little utilised, are *in situ* procedures. Studies following this methodology can be conducted directly in water reservoirs, minimising artefacts during sampling and storage, as well as avoiding any disturbance in the equilibrium of the original system.

Consequently, the problems involved in the study of metal reactivity in aquatic system cannot be solved simply by extremely sensitive techniques, but require new procedures which allow an *in situ* metal speciation. This work presents a new *in situ* technique based on a metal-chelator (DM-Chelator) consisting of cellulose bags filled with tetraethylene-pentamine-hexaacetate (TEPHA) resin. They are directly introduced into the water body to characterise the lability of aqueous metal species as a function of time and of the quantity of the exchanger, providing more realistic information about the reactivity of metals in water systems.

2. Experimental

2.1 *Chemicals and reagents*

All the reagents used were of high-purity grade unless otherwise stated. Diluted acid and alkaline solutions necessary were prepared by diluting 30% hydrochloric acid

Table 1. Characteristics of water samples collected from a tributary of the Itapanhaú (Feb 2006) and Ribeira de Iguape rivers (Oct 2005).

Parameters analysed	Water samples	
	Itapanhaú	Ribeira de Iguape
pH	4.4	4.0
Temperature (°C)	23.0	19.6
Conductivity ($\mu S\,cm^{-1}$)	39.0	169.0
Dissolved oxygen ($mg\,L^{-1}$)	2.2	3.1
Total organic carbon ($mg\,L^{-1}$)	15.3	38.5
Absorbance at 436 nm (A)	0.085	0.23
Molar absorbance (A L/cm mol C)	66.7	70.5

(suprapure, Merck AG, Germany) or sodium hydroxide-monohydrate (suprapure, Merck AG, Germany) in high-purity water (resistivity $18.2\,M\Omega\,cm^{-1}$ at 25°C, Milli-Q system, Millipore, USA).

2.2 *Water sample characterisation*

The pH, temperature, conductivity and dissolved oxygen were determined *in situ* after calibration of the equipment, using standard reference solutions. In the laboratory, the water samples were digested with concentrated HNO_3 heated to 120°C and the total metal concentrations of Cu, Pb, Mn and Ni were determined by atomic absorption spectrometry. The Total Organic Carbon (TOC) contained in water was determined by catalytic combustion in an oxygen stream and subsequent IR detection with a Schimadzu TOC 5000 analyser [15]. Table 1 lists the analyses of the water samples under study.

2.3 *Preparation of the DM-TEPHA system*

The system was prepared using small cellulose bags (tea bags) pre-purified with solutions of sodium hydroxide and chloric acid, each $0.1\,mol\,L^{-1}$, packed with TEPHA (tetraethylene-pentamine-hexaacetate, Fluka, Germany) resin. After it was sealed, the DM-TEPHA system was immersed *off site* in solutions containing aquatic humic substances extracted and doped with known concentrations of metals ions under controlled conditions and an evaluation was made of the influence of complexing time and concentrations of AHS and metals on the performance of the system. To ascertain the influence of the parameters of interest on the performance of the DM-TEPHA system, experiments were conducted in laboratory varying the pH value, reaction time, metal concentration and AHS, respectively. By these experiments the DM-TEPHA system was optimised for its subsequent application in *in situ* experiments as previously described in Rosa *et al.*'s work [13].

2.4 In situ *application of the DM-TEPHA system*

The DM-TEPHA system was prepared as already described and separated into groups, each containing 4 bags with 0.25, 0.5, 1.0 and 2.0 grams of TEPHA resin. Each system was wrapped in tulle and tied with string inside a support. Each group was then immersed in the river water and left there for a given time. The immersion times were 30, 60, 120, 240 and 480 min.

The DM-TEPHA systems were applied *in situ* in humic-rich tributaries of the Ribeira do Iguape river (Iguape – São Paulo – Brazil) GPS and in the tributary of the Itapanhaú river (Bertioga – São Paulo – Brazil) GPS. At the end of each reaction period studied, the TEPHA resin was removed from the cellulose bags and the metals eluted on site by agitation with 10 mL of suprapure HNO_3, 1.0 mol L^{-1}, for 30 min, followed by siphoning.

2.5 *Metal determinations by AAS*

The metals were determined in the laboratory using a Zeeman electrothermal atomic absorption spectrometer (Varian, Model Spectra AA240Z) equipped with an autosampler (Varian, Model PSD 120) coupled to a Dell PC. The spectrometer's graphite tubes were designed with an integrated platform. Argon 99.99% (White Martins, Brazil) was used as the purge gas. Each standard and sample solution was measured in triplicate. Synthetic metal standards with the same acid concentration as the experimental samples were used for calibration.

3. Results and discussion

3.1 In situ *application of the DM-TEPHA system*

The metals present in AOM-M complexes can be classified as labile or inert depending of the stability of the complexes formed. Inert metals have high stability with AOM while labile metals are those that in some special conditions, like variation of pH, ionic strength, or redox potential, can be available to the aquatic system. The determination of inert and labile metals in aquatic systems is not an easy analytical task and generally requires a combination of techniques for complementation and/or confirmation of the results. Dissolved and bonded forms of metals in aquatic systems can be represented by hydrated ions of free metals and complexed ones [11]. In this study labile fraction refers to the portion of the retained metal on a chelating resin which contains the free metal and the very or moderately labile metal determining in that manner the labile fraction in relation to the exchanger.

In principle, the metal exchange between resin (TEPHA) and the labile metal fraction bound to macromolecular Aquatic Organic Matter (AOM-M) can be described by the following equilibria in:

$$\text{AOM-M} \overset{K_{AOM}}{\rightleftharpoons} \text{AOM} + \text{M} + \text{IER} \overset{K_{IER}}{\rightleftharpoons} \text{M-IER}$$

where AOM-M indicates the species formed between metal ions and AOM, IER indicates ion exchange resin (solid phase), and M-IER indicates the species formed between metal ions and the chelating exchange resin.

Hence, the concentrations of AOM-M and M-IER species, which are governed by their respective complexation constants K_{AOM} and K_{IER}, can shift to AOM-M as a function of an AOM increase. This important parameter discussed in the literature [16–18] strongly influences the lability of metal fractions complexed by AOM.

In addition, distribution complexation constants K_{IER} of free metal ions M between an aqueous solution and chelating IER, Equation (1),

$$K_{IER} = \frac{[M - IER]}{[M][IER]} \tag{1}$$

can also be used, to determine the concentration of free metal ions [M] in the humic-rich water samples under study [17]. In the case of ion exchangers and low metal loadings such distribution equilibria are often simplified [17] by distribution coefficients K_d, Equation (2)

$$K_{IER}[IER] = K_d = \frac{[M - IER]\,\text{mmol/g}}{[M]\,\text{mmol/mL}} \tag{2}$$

In contrast to uniform binding groups of chelating ion exchangers such as IER, however, AOM generally present a wide spectrum of functional groups and can interact with various metal ions, forming complexes of different stabilities. When the metal/mass ratio is low, metal ions preferentially bind to functional groups that favour the formation of complexes of maximum thermodynamic stability [16].

As can be seen (Figure 1), stationary exchange equilibria obtained between labile metal fractions in Ribeira de Iguape river and immersed TEPHA resin bags were obtained within 100 min. The order of complexation of the metals found by an DM-TEPHA system containing 2.0 grams of exchanger was: Fe > Cu > Mn > Ni > Pb, indicating that Fe, Cu and Mn presented a higher relative lability due to their greater affinity for the TEPHA resin. On the other hand, Ni and Pb are presumably more strongly linked to AOM, thus presenting lower relative lability.

Figure 2 shows the relative lability of Cu, Mn, Ni, Pb and Fe present in the Itapanhaú river, *in situ* by the DM-TEPHA system. As can be seen, the stationary exchange equilibria between Mn and TEPHA resin were obtained within 25 min, whereas the other metal ions

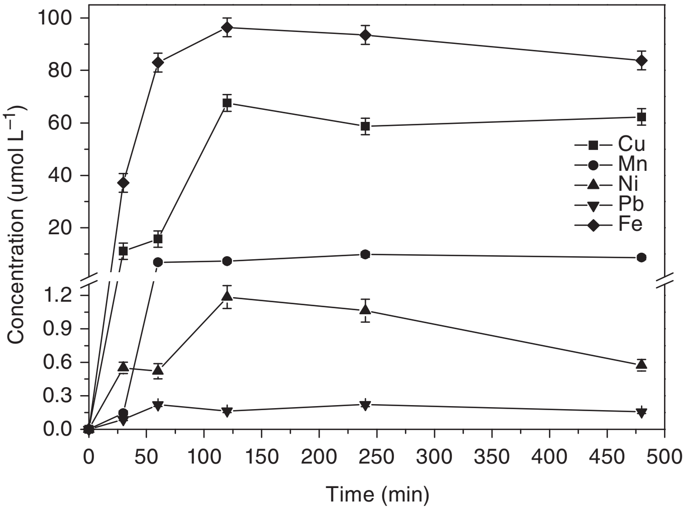

Figure 1. *In situ* separation of labile metal fractions in river water by immersed TEPHA bags. Conditions: pH 4.0, DM-TEPHA system containing 2.0 g of exchanger, tributary of the Ribeira de Iguape river. The experiments were performed in triplicate.

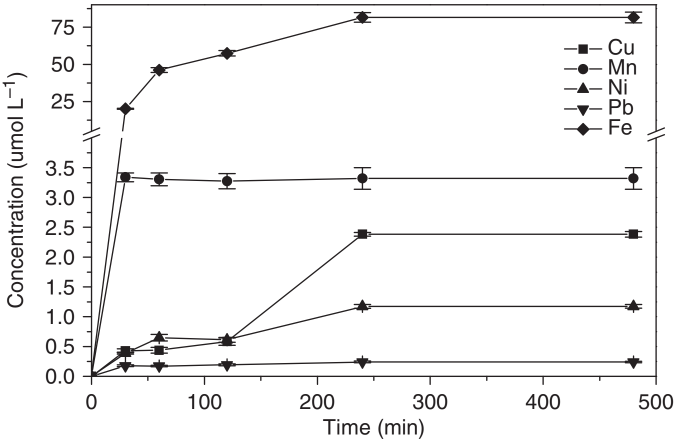

Figure 2. *In situ* separation of labile metal fractions in river water by immersed TEPHA bags. Conditions: pH 4.4, DM-TEPHA system containing 2.0 g of ion exchanger, tributary of the Itapanhaú river. The experiments were performed in triplicate.

require up to 250 min. The order of complexation observed for the system containing 2.0 g of TEPHA resin was Fe > > Mn > Cu > Ni > Pb. Using the DM-TEPHA system, it was found that there were practically no alterations in the initial concentrations of the Mn and Pb species as a function of time, indicating the equilibrium of the AOM-Mn and AOM-Pb complexes in relation to the TEPHA-Mn and TEPHA-Pb complexes. It was found that, although the water bodies studied present distinct physicochemical and structural characteristics [13], the order of relative lability of the metals to the DM-TEPHA system showed similarities, with an inversion between the order of Cu and Mn.

Comparing the relative order of lability of the metals present in the tributaries by DM-TEPHA system with a similar one, containing cellulose modified with p-aminobenzoic (DM-Cell-PAB) in previous studies [19], the order of complexation of the metals found in the tributary of Ribeira de Iguape river containing 1.0 g of exchanger was Fe > Mn > > Ni > Cu > Pb and the tributary of Itapanhau river was Fe > Mn > > Pb > Cu > Ni. For the DM-Cell-PAB system, the order of complexation presented differences in microconstituent elements. These results are interesting, showing that the lability of metals in aquatic systems is not only highly dependent on the body of water and characteristics of the AOM but mainly on the reactive group that will interact/compete with the metal species in the environment.

It used 1.0 g of Cell-PAB exchanger because it is more voluminous than TEPHA resin and the packing of the exchanger is inefficient. The size of the ‘tea bags’ becomes the limiting factor (4.0 × 5.0 cm) of the mass of the resin. However, larger cellulose bags can be elaborated for this purpose.

The study of the influence of the exchanger’s mass on the lability of metal ions as a function of time can provide important information about the kinetics, complexing rate constant, and the possible order of the reaction. Figure 3 exemplifies the relative lability of Fe ions for the DM-TEPHA systems.

An analysis of the behaviour of Fe ions indicates an increase in the complexation of Fe by the TEPHA exchanger as the resin mass is increased. Obviously, this behaviour is associated with the increase in the number of accessible sites of the exchanger in response

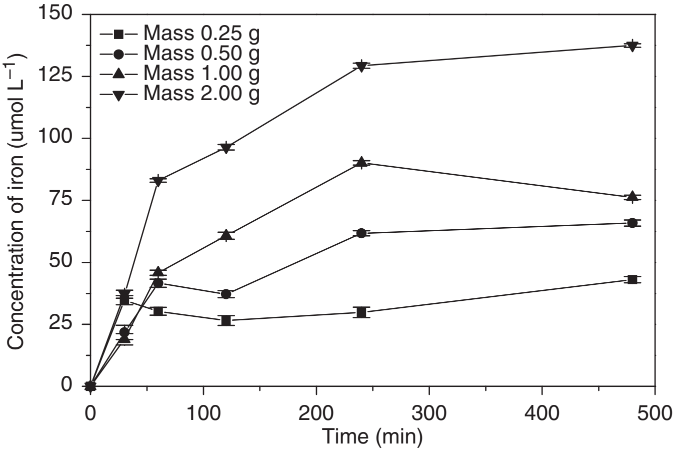

Figure 3. Separation of labile iron by the DH-TEPHA system as a function of time and increasing TEPHA amounts. Conditions: systems containing 0.25, 0.50, 1.0 and 2.0 g of ionic exchanger, tributary of the Ribeira de Iguape river. The experiments were performed in triplicate.

to the increase of its mass. However, over time, the 0.25 g mass proved inefficient in increasing the quantity of Fe complexed, indicating that, with 0.25 g of TEPHA resin, all the reactive sites were saturated after 30 min of contact between the DM-TEPHA system and the river water. Stationary exchange equilibrium of the exchange occurred within 240 min for the remaining metal species.

From a study of the influence of the exchanger's mass, one can also estimate the rate constant and the order of the exchange reaction with TEPHA resin – parameters that provide information about the reactivity of different metal species in water bodies. These parameters can be estimated from plotting a graph of the initial exchange velocity versus the time of contact of the DM-TEPHA system, which gives one the different values of the initial rate of complexation for different exchanger masses. Based on the graph of the initial complexation rate versus the different exchanger masses, one then use the tangent of the straight line to determine the exchange rate constant ($k - \mathrm{L}^{-1}\mathrm{min}^{-1}$), as indicated in Figure 4.

As can be seen in Figure 4, the initial complexation rate is directly proportional to the mass of resin of the system studied here, allowing one to infer that the competition of Fe ions follows a first order reaction. The value of correlation coefficient is $r = 0.94$ which is an indication that other influential factors are involved in a given process. This value is acceptable due to the large complexibility of an environmental matrix [20].

Figure 5 depicts the complexation of manganese by the DM-TEPHA system. Accordingly, 0.25 g of TEPHA resin was not sufficient for a satisfactory Mn exchange over time. Maximum Mn complexation on TEPHA resin was achieved within 240 min.

Figure 6 presents a kinetic treatment of Mn ion exchange by the two systems studied here, indicating a second order exchange reaction of Mn ions with the DM-TEPHA system, and showing that the concentration of exchanged metal is rapid at the beginning of the process, declining as a function of contact time with the river water.

Considering that Figure 6 already indicates second order kinetics, the graph of the complexation rate was plotted as a function of the square of the mass of resin to obtain

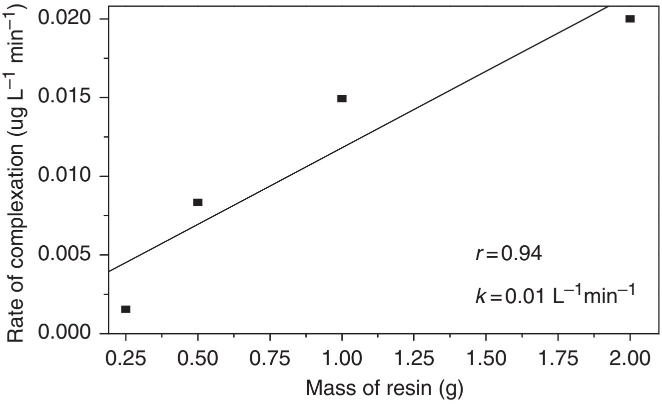

Figure 4. Determination of the exchange rate constant and exchange order of iron ions by the DM-TEPHA system. Conditions: systems containing 0.25, 0.50, 1.0 and 2.0 g of ion exchanger, tributary of the Ribeira de Iguape river.

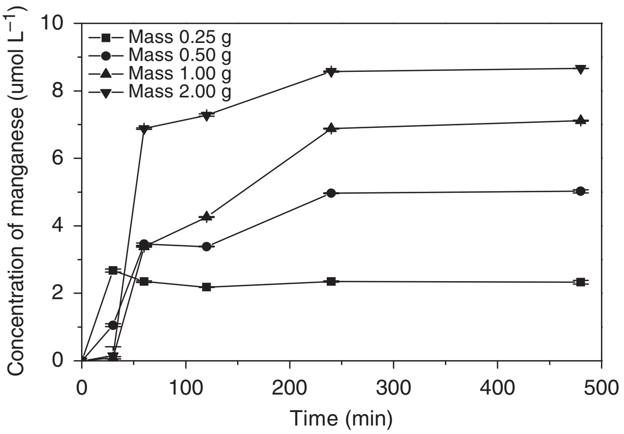

Figure 5. Separation of labile manganese in river water by DM-TEPHA system as a function of time and increasing TEPHA amounts. Conditions: systems containing 0.25, 0.50, 1.0 and 2.0 g of TEPHA, tributary of the Ribeira de Iguape river. The experiments were performed in triplicate.

the linearisation. Figure 7 confirms the hypothesis of second order kinetics for the exchange of the Mn species with the DM-TEPHA system. Based on the k values, it was found that the velocity of the exchange of the Mn species of the river water with the DM-TEPHA system was $k = 3.15 \times 10^{-3}\,\mathrm{L^{-1}min^{-1}g^{-1}}$.

Table 2 summarises the results obtained for equilibrium times, velocity constants and order of the exchange reactions for the metals (Fe, Mn, Cu, Pb and Ni) by the DM-TEPHA systems in the rivers studied here (tributaries of the Ribeira de Iguape and Itapanhaú rivers).

Table 2 indicates that, in most cases, it was possible to assess the stationary equilibrium time, rate constant and order of the reactions involved in the process of competition of the metal species present in the water bodies with the reactive groups of the ion exchanger contained in the system.

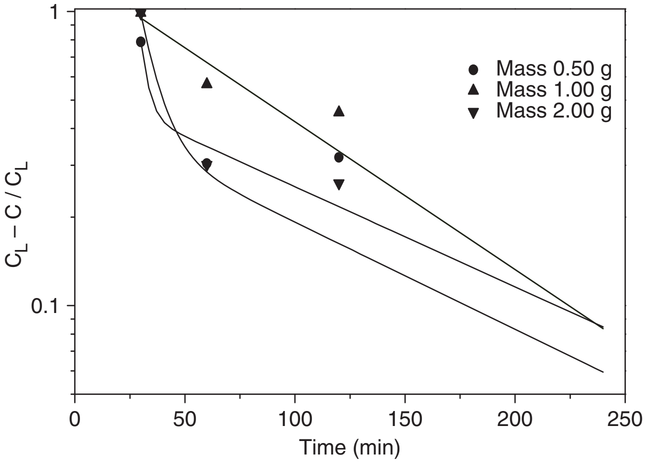

Figure 6. Kinetic study of the exchange between labile manganese (C_L – ion concentration in the equilibrium and C – ion concentration in the aliquot) and the DM-TEPHA system. Conditions: systems containing 0.25, 0.50, 1.0 and 2.0 g of ionic exchanger, tributary of the Ribeira de Iguape river.

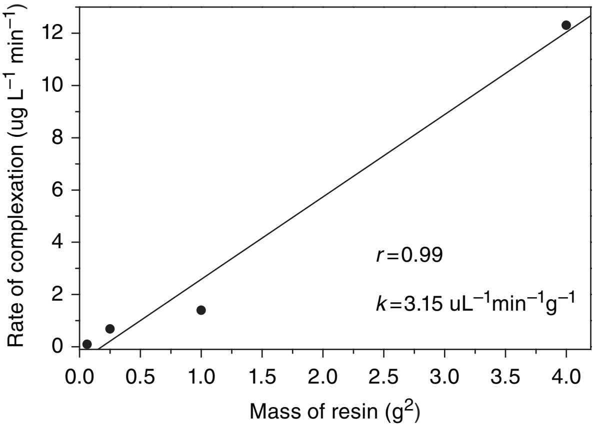

Figure 7. Determination of the exchange rate constant and exchange order of manganese separation by the DM-TEPHA system.

The DM-Exchanger system reached stationary exchange equilibria more rapidly with metal ions in the Ribeira de Iguape water than with those contained in Itapanhaú. In the former, all the metals reached exchange equlibria within 120 min, except Fe (240 min). In the Itapanhaú, the exchange kinetics of the studied metals was slower, with stationary equilibria times varying from 240 min for Fe, Cu and Ni to 120 min for Mn and Pb. Table 2 also shows variations in the values of the exchange rate constant and in the order of the exchange of the metal species. The reactions were mostly of the first order, indicating that the exchange of metal species from liquid to solid phase was directly proportional to mass of the exchanger employed in the DM-TEPHA system. These results are attributed to the differences in the structural and physicochemical characteristics of the AOM present in the two water bodies studied.

Table 2. Stationary equilibrium time, rate constants, and order of the exchange reactions of metal species in the tributaries of the Ribeira de Iguape and Itapanhaú rivers using the DM-TEPHA system.

Water body	Metal	Equilibrium time (min)	Rate constant	Order of the reaction
Tributary of the Ribeira de Iguape	Fe	240	0.01	1st
	Mn	120	3.15	2nd
	Cu	120	–	2nd
	Ni	120	0.37	1st
	Pb	120	–	–
Tributary of the Itapanhaú	Fe	240	8.01	1st
	Mn	120	0.01	1st
	Cu	240	–	–
	Ni	240	0.13	–
	Pb	120	–	–

Therefore, the DM-TEPHA analytical procedure can be applied not only *in situ* to differentiate labile and inert species in the aquatic system, but also to estimate the relative lability of metal species under study and to obtain interesting information about the exchange kinetics and stability of the complexes present in water bodies.

3.2 *Environmental relevance of the results obtained in terms of the lability of metal species in water bodies*

In addition to the development of the DM-TEPHA procedure, which is applicable *in situ* to characterise the interactions between metal species in aquatic systems, it is also possible to obtain a labile fraction of metal species in those systems. The theoretical fundaments involved in the proposed Equation (3) shown below were published in the work of Goveia *et al.* [19].

$$C_{ML} = \frac{C_{MR} \times V_{\mathrm{e}}}{v \times (A_S \times m_r) \times t} \tag{3}$$

where: C_{ML} = concentration of labile metal (μmol L^{-3}), C_{MR} = concentration of retained metal (μmol L^{-1}), V_{e} = volume of acid used in the elution of the metal retained in the exchanger (L), v = velocity of the flow of the river ($\mathrm{m\,s^{-1}}$), A_s = surface area of the exchanger ($\mathrm{m^2\,g^{-1}}$), m_r = mass of the exchanger (g), t = immersion time of the DM-Exchange system in the water body (s).

Considering that 120 min (Table 3) was the average time for the metals to reach a stationary exchange equilibrium with the DM-TEPHA system, our calculations were based on an immersion time equivalent to 60 min. Thus, from data of the surface area ($15\,\mathrm{m^2\,g^{-1}}$) and resin mass (2.0 g), river water flow velocity ($0.95\,\mathrm{m\,s^{-1}}$ for the Ribeira de Iguape and $1.43\,\mathrm{m\,s^{-1}}$ for the Itapanhaú).

It should be noted that due to their high concentrations, greater quantities of Fe and Mn ions may also be present in free form, rendering them more accessible to the TEPHA exchanger. However, the concentration of total Cu is lower than the concentration of Mn,

Table 3. Concentrations of total metal and labile metals, based on an immersion time of 60 min of the DM-exchange systems in the tributaries of the Ribeira de Iguape and Itapanhaú rivers.

			DM-TEPHA			DM-Cell-PAB [19]		
Water body	Metal	Total Metal (µmol L^{-1})	*C$_{MR}$ (µmol L^{-1})	C$_{ML}$ (10^{-4}µmol L^{-1})	Kd$_{cond}$ (L kg^{-1})	*C$_{MR}$ (µmol L^{-1})	C$_{ML}$ (10^{-4}µmol L^{-1})	Kd$_{cond}$ (L kg^{-1})
Tributary of the Ribeira de Iguape	Fe	19.47 ± 0.04	82.99	80.93	21.3	62.13	179.05	31.9
	Mn	7.83 ± 0.04	6.88	6.72	4.4	10.54	30.94	13.46
	Cu	$1.40 \times 10^{-2} \pm 0.01$	15.70	15.31	5607.1	1.03	3.04	735.7
	Ni	0.60 ± 0.01	0.52	0.51	4.3	1.09	3.17	18.2
	Pb	$3.70 \times 10^{-2} \pm 0.02$	0.22	0.21	29.7	0.10	0.30	27.0
Tributary of the Itapanhaú	Fe	16.28 ± 0.07	46.09	29.90	14.2	204.87	397.49	125.8
	Mn	4.55 ± 0.11	3.29	2.15	3.6	86.91	168.55	191.0
	Cu	$1.30 \times 10^{-2} \pm 0.01$	0.44	0.28	169.2	0.84	1.64	646.2
	Ni	0.53 ± 0.01	0.65	0.42	6.0	1.07	2.08	20.2
	Pb	$3.50 \times 10^{-2} \pm 0.02$	0.17	0.11	22.9	0.24	0.46	68.6

*C$_{MR}$: Concentration of metal retained in the exchanger.

but as it is a continuous flow, it is possible to observe that the ions Cu have more relative lability than do the ions Mn, indicating that only information about the total concentration is not sufficient to assess the bioavailability of the element, it is necessary the speciation.

Comparing the results obtained by both systems (DM-TEPHA and DM-Cell-PAB) the concentrations of complexed metals to the DM-Cell-PAB system were higher [19]. In other words, the system has more competition by present metals in tributaries than the DM-TEPHA exchange. The present ions in Ribeira de Iguape river presented more relative lability to DM-TEPHA system than the present ions in Itapanhau river. The DM-TEPHA was more efficient in the differentiation of labile fraction of the Cu ions, while DM-Cell-PAB was more efficient for the ions Ni, Pb and Fe.

This behaviour is associated with the different affinities of the metals for the functional groups of the exchanger used in each case, which will compete with the functional groups of the aquatic organic matter in the complexation of metal species. The Cell-PAB resin (cellulose organo-modified with p-aminobenzoic groups) has a lower content of polarisable groups than TEPHA resin (tetraethylene-pentamine-hexaacetate), which has six acetate groups present. Consequently, Cell-PAB represents a harder base than TEPHA resin. This is shown in the results, with Fe and Mn (hard acids) and Pb and Ni (intermediary acids) showing higher affinity for DM-Cell-PAB compared to the DM-TEPHA system. Hence, selection of the exchanger to be used in the system will depend on the metal species to be studied

The values of C_{ML} yield quantitative data about the reactivity of species in aquatic systems, supplying information for better understanding the processes taking place in those systems compared to simple information on total or relative lability concentration of metals in the water body under study. The C_{ML} values yield quantitative data on the reactivity of species in aquatic systems, supplying information that provides a better understanding of the processes taking place in these systems, compared to that obtainable from simple measurements of total concentrations or relative labilities of metals in the water body under study. Specific exchangers can be used in the DM-exchanger system, depending on the relative lability to be evaluated. The results showed that, for the metals studied, the Cell-PAB exchanger was more efficient than TEPHA resin. However, to study other metals, such as Cd(II), Ag(I), Hg(I) and Au(I), which can be considered soft acids, the system containing the TEPHA exchanger may be more suitable [21]. The results showed that for the metals studied the Cell-PAB exchanger was more efficient than TEPHA resin. However, to study other metals, such as Cd(II), Ag(I), Hg(I) and Au(I), which can be considered soft acids, the system containing the TEPHA exchanger could be considered more suitable.

Concerning the use of the TEPHA exchanger in this work, compared to the DM-Cell-PAB system previously proposed, the TEPHA resin has already been used in the laboratory to study the lability of metals, because it can reproduce more effectively the process of (bio-)availability of metals in water and soils. Consequently, the results obtained using this exchanger can provide a better understanding of the behaviour of metals in aquatic systems than those obtained using the Cell-PAB exchanger.

Values of the Kd constant were determined, and the results included in Table 3. For DM-TEPHA, we could obtain the following estimation of so-called 'conditional' Kd values for this sample: $Kd_{cond} =$ complexed metal in the resin ($\mu mol\,kg^{-1}$)/total metal in the river ($\mu mol\,L^{-1}$). These values were calculated and are provided in Table 3. In the case of Fe, a major fraction of this metal seems to be exchange-inert, presumably due to strong binding to humic and inorganic colloids.

Multi-stage separation techniques (e.g. exchanger columns) enable the separation of 'available/labile' metal fractions from solutions, given sufficient contact times. The differences between the ion exchangers are not large, and are due to the different Kd values of the metal ions studied.

4. Conclusions

The DM-TEPHA system optimised in the laboratory and employed in field experiments is a simple methodology enabling an *in situ* differentiation of label/inert metal species. This methodology requires only low amounts of reagents avoiding time-consuming on-site sampling and sample preparation. The *in situ* application of the DM-TEPHA system in two distinct water bodies supplied important information about the relative lability of heavy metals in Brazilian rivers under environmental study.

The TEPHA resin has been used to study metal lability because this exchanger can reproduce more efficiently the process of (bio-)availability of metals in aquatic systems. Hence, this exchanger is more suitable for use in studies of metal lability in aquatic systems than Cell-PAB (previously studied). The study undertaken with two different exchangers enabled confirmation that the relative metal lability will necessarily depend on the reactive group of the exchanger used. This demonstrates the versatility of the system, since different exchangers can be employed, depending on the nature of the metals present.

Acknowledgements

Financial support of this work by FAPESP, CNPq, CAPES and FUNDUNESP (Brazilian Agencies) is gratefully acknowledged.

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