Talanta 84 (2011) 287–292

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Evaluation of cadmium species lability using ion-pair reversed phase HPLC coupled on-line with inductively coupled plasma mass spectrometry

Hua Yan[a], Limin Yang[a], Qiuquan Wang[a,b,*]

[a] Department of Chemistry & the Key Laboratory of Analytical Sciences, College of Chemistry and Chemical engineering, Xiamen University, Xiamen 361005, China
[b] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China

ARTICLE INFO

Article history:
Received 11 November 2010
Received in revised form
23 December 2010
Accepted 6 January 2011
Available online 15 January 2011

Keywords:
Cadmium species lability
Bioavailability
Phaeodactylum tricornutum
ICP-MS
Ion-pair reversed phase HPLC

ABSTRACT

Inductively coupled plasma mass spectrometry (ICP-MS) coupled on-line with an ion-pair reversed phase HPLC (IP-RP HPLC) was developed for determining the lability of Cd species. The IP-RP HPLC–ICP-MS system measures chromatographic behaviors of Cd species in the presence of different model complexing agents (L) with stability constants ($\log K_{CdL}$) from 3.8 to 19.0. Cd species with $\log K_{CdL}$ higher than 16, between 8 and 16, and smaller than 8 was then classified into *inert*, *moderately labile*, and *labile species*, respectively. The conditional stability constants and dissociation rate constants were also estimated from their corresponding chromatographic behavior. This method was applied to evaluating the lability-dependent biouptake of different Cd species in *Phaeodactylum tricornutum*, a typical unicellular marine diatom. IP-RP HPLC–ICP-MS is a useful and promising technique for determining the lability of noncovalent-bonded metal species (such as Cd species) in the environment and for forecasting their corresponding bioavailability especially when their speciation cannot be rigorously controlled and measured.

1. Introduction

The bioavailability and/or toxicity of an element to organisms as well as its mobility in the environment depend strongly on the types of its species present [1,2]. In many cases, a labile species relates strongly to its bioavailability and/or toxicity [3–6]. Under dynamic conditions such as those in natural aquatic systems, knowledge of dynamic compound speciation and metal lability is fundamental for establishing a relationship between metal speciation and bioavailability and/or toxicity [7,8]. Metal lability describes the ability of a species to maintain equilibrium with the free metal ion (M) within the context of an ongoing interfacial process in which a particular species is consumed. Metal lability is an operationally defined parameter that depends on the effective time scale of the analytical technique used and association and dissociation rate constants of the formation reaction between M and the coexisting ligand (L) of ML complex [9].

Several methods have been proposed to estimate the lability of metal species. For examples, voltammetric methods provide useful information on the lability of metals, metal-binding ligand concentrations, complexation capacity, and complex stability constants [10,11]. DGT (diffusive gradients in thin films) technique has been used to quantitatively measure free and labile metal species in aquatic environments [12,13]. Furthermore, chromatographic technique based on the interaction of metal species with a stationary phase coupled off-line and/or on-line with an element-specific detection technique has been utilized for elemental speciation, especially for covalent-bonded and thermodynamically stable species such as metallic organic compounds [14–21].

In environmental samples with highly concentrated organic matters, noncovalent-bonded metal species are easily formed. Compared with thermodynamically stable metal species, noncovalent-bonded metal species is not associated through covalent bonds and are in chemical disequilibrium with respect to the initial equilibrium composition and tends to move towards a new chemical equilibrium. These thermodynamically unstable species change to some extent during the separation process and the experimental results obtained would be somewhat in disagreement with the distribution from theoretical thermodynamic equilibrium calculations [3,22–25]. Moreover, if the target species of a metal achieves a new equilibrium fast enough before detection, the peak appearing in the chromatogram will not reflect the original speciation [26–28]. Strongly acidic sulfate, weakly acidic carboxyl, and amido are typical groups on the surface of microor-

* Corresponding author at: Department of Chemistry & the Key Laboratory of Analytical Sciences, College of Chemistry and Chemical Engineering, Xiamen University, 422 South Siming Road, Siming District, Xiamen 361005, Fujian, China.
Tel.: +86 592 2181796; fax: +86 592 2187400.
E-mail address: qqwang@xmu.edu.cn (Q. Wang).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.01.019

ganism membrane. Ion exchange is the most possible mechanism involved in the metal biosorption process [29]. The distribution behavior of an elemental species between the stationary and mobile phases during ion exchange chromatographic separation process may be considered to be similar to those between heterogeneous interfaces in an environmental and/or biological system. However, few studies [27,30] have been reported so far concerning such an important issue that can be expected to offer valuable information for evaluating the bioavailability and/or toxicity of an element to organisms as well as its mobility in the environment.

In this work, Cd, a typical kinetically fast and toxic metal, was chosen as an example in order to investigate the lability of noncovalent-bonded metal species using an ion-pair reversed phase HPLC (IP-RP HPLC) coupled online with inductively coupled plasma mass spectrometry (ICP-MS). Lability of the Cd species formed with various model anthropogenic and/or natural occurring ligands, which are likely to exist in the environment, during the separation process of IP-RP HPLC were systematically investigated using ICP-MS for the specific detection of Cd. Based on their chromatographic behavior and thermodynamic stabilities, three types of Cd species of *labile*, *moderately labile*, and *inert* were operationally defined. This methodology was then applied to study the lability-dependent bioaccumulation of Cd species in a typical marine diatom, *Phaeodactylum tricornutum*.

2. Materials and methods

2.1. Chemical reagents

All reagents used were at least of analytical grade and Milli-Q water (18 MΩ cm) from a Milli-Q water purification system (Millipore, USA) was used throughout. Sodium dodecylsulfate (SDS) from TCI was used as an ion-pair reagent (IPR) to modify the ODS column. Cd stock solution (100 μg g^{-1}) was from National Research Center for Certified Reference Materials of China. Humic acid (HA) from Sigma (Sigma–Aldrich, Germany) was dissolved in water and filtered over a 0.45 μm membrane filter. Diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid disodium salt (EDTA–Na_2), nitrilotriacetic acid (NTA), citrate acid (Cit), N-(2-hydroxylethyl)ethylene diaminetriacetic acid (HEDTA), N-(2-hydroxylethyl)-iminodiacetic acid (HIDA), and 8-hydroxyquinoline-5-sulphonic acid (HQS) were purchased from TCI, Japan, and pyridine-2,6-dicarboxylic acid (PDCA) purchased from Fluka, Switzerland were used as model ligands and 0.01 mol L^{-1} stock solutions of these ligands were prepared by dissolving the corresponding solid in water, respectively. Solutions containing 5.0×10^{-6} mol L^{-1} Cd and the selected model ligand of different concentrations were mixed together to form Cd species. The pH values of these solutions were adjusted between 5.5 and 7.5 by 50 mmol L^{-1} buffer solution of NH_4Ac–HAc or NH_4Ac–$NH_3{\cdot}H_2O$, and then allowed to equilibrate for 24 h prior to IR-RP HPLC–ICP-MS analysis. Species distribution in the solution was calculated using a home-edited computer program YLM 2.0, and thermodynamic stability constants ($\log K_{CdL}$) of possible species was obtained from NIST critical selected stability constants database version 6.0 [31].

2.2. Instrumentation

A quadruple ICP-MS (Agilent 7500 ce, Agilent Technologies, USA) equipped with a micro flow nebulizer, a ShieldTorch system, a spray chamber cooled by a Peltier system, and an octopole reaction system was used for cadmium determination. HPLC system used for cadmium speciation consisted of an Agilent 1100 pump (Agilent Technologies, USA) fitted with a six-port injection valve (model 7725i, Rheodyne, Rohner Park, CA, USA) with a 20 μL injection loop and a reversed-phase analytical column (Kinetex ODS column, 4.6 mm × 100 mm; pore size 100 Å; particle size 2.6 μm, Phenomenex). The commercial reversed-phase ODS column was modified with SDS for the separation of Cd species. The column was coated by passing solution of 1 mmol L^{-1} SDS at 0.3 mL min^{-1} for 15 h at 25 °C, and the mobile phase was then switched to water at 0.2 mL min^{-1} for 30 min [32]. Ammonium acetate (NH_4Ac) solution of 0.1 mol L^{-1} was chosen as a mobile phase, and pH values were adjusted to 5.5, 6.0, 6.5, 7.0, and 7.5 using glacial acetic acid and/or ammonia solution. The elution of SDS was examined by repeated injection of cadmium ion before and after injection of other Cd species. If the retention time of cadmium ion changed, the column was washed with methol/H_2O (70:30, v/v), and modified SDS again. The HPLC was connected to ICP-MS using 20 cm of 0.025 cm i.d. Teflon tube. Replicate injection of each model solution was made onto the column. Online signal of cadmium isotope ^{111}Cd was determined by ICP-MS and the quantification was performed in time-resolved analysis mode. The optimized operation conditions and data acquisition parameters are summarized in Table 1.

Table 1
Operational parameters of the IP-RP HPLC–ICP-MS system.

ICP-MS parameters	
RF power	1500 W
Sampling depth	8.0 mm
Carrier gas	0.80 L min^{-1}
Makeup gas	0.26 L min^{-1}
Acquisition mode	Time resolved analysis
Isotope monitored	^{111}Cd
Dwell time per point	200 ms
Replicates	3
HPLC conditions	
Column	Phenomenex Kinetex ODS (4.6 mm × 100 mm, 2.6 μm) modified using SDS
Mobile phase	0.1 mol L^{-1} ammonium acetate
Elution mode	Isocratic mode
Flow rate	0.7 mL min^{-1}
Injection volume	20 μL

2.3. Cd bioaccumulation in marine diatom P. tricornutum

P. tricornutum were obtained from the Center for the Collection of Marine Bacteria and Phytoplankton in the State Key Laboratory of Marine Environmental Science at Xiamen University. The growth medium was natural seawater containing f/2 enrichment culture solution (f/2 solution contains major nutrients, including nitrate, phosphate, silicate, essential trace elements, and vitamins) [33,34]. *P. tricornutum* were collected in their late exponential growth phase (ca. 2×10^5 cells mL^{-1}) by centrifugation at 4000 × *g*, and further resuspended in culture solutions. The pH value of each culture solution was adjusted to 6.0 using 0.01 mol L^{-1} 2-morpholinoethanesulfonic acid. The culture solutions for Cd stress experiments contain 4.5×10^{-5} mol L^{-1} Cd and 4.5×10^{-4} mol L^{-1} EDTA, HEDTA, HIDA, PDCA, NTA, HQS, and Cit, respectively; the concentration of HA was 5 mg L^{-1}. After 40 min of exposure, aliquots (10 mL each) of the algal suspension were filtered through 0.45 μm nitrocellulose filter, and filtered algal cells were washed initially with 5 mL natural seawater three times, and then with 10 mL EDTA (0.01 mol L^{-1} at pH 6.0) to remove the weakly surface-bound Cd [35]. Internalized Cd in the cells was determined after digestion in 0.5 mL concentration HNO_3 (Suprapurity, Merck). Internalized and surface-bound Cd were determined using ICP-MS. Cell densities were determined using a spectrophotometer at 625 nm wavelength. Cell numbers were obtained from the linear relationship between the optical density and the cell numbers counted using microscopy [36,37].

3. General concept

The simplest form of interaction between a free Cd ion and a ligand (L) in an aqueous solution can be illustrated as

$$Cd + L \xrightarrow{K_{CdL}} CdL \quad (1)$$

The stability constant of Cd complex can be expressed as $K_{CdL} = k_a/k_d$, where k_a is the association rate constant ($L\,mol^{-1}\,s^{-1}$) and k_d the dissociation rate constant (s^{-1}).

Coating an ordinary ODS column with an IPR is known to produce an effective stationary phase for ion exchange chromatography [32,38]. When a sample is injected onto the column, the following interaction between the free Cd ion in the sample and the functional group of the IPR modified on the stationary phase will occur:

$$IPR + Cd \xrightarrow{K_{Cd-IPR}} Cd\text{–}IPR \quad (2)$$

where K_{Cd-IPR} is the association constant of the interaction between the free Cd ion and the IPR. Such interactions result in the initial equilibrium shown in Eq. (1) being perturbed when the possible side reactions between the free Cd and the ligands in the mobile phase were ignored. If the consequent dissociation of the complex ($CdL \rightarrow Cd + L$) can be assumed to be first order kinetics with the rate constant k_d, the dissociation degree can be expressed as:

$$[CdL]_t = [CdL]_0 e^{-k_d t} \quad (3)$$

and thus

$$\ln \frac{[CdL]_0}{[CdL]_t} = k_d t \quad (4)$$

where $[CdL]_t$ is the Cd complex concentration ($mol\,L^{-1}$) remaining after time t (s) and $[CdL]_0$ is the initial Cd complex concentration ($mol\,L^{-1}$) at injection time $t = 0$. The dissociation rate constant k_d of CdL can be determined from the slope of the plot of $\ln([CdL]_0/[CdL]_t)$ against t according to Eq. (4).

The amount of a complex that dissociates during the process of chromatographic separation is determined by the rate of dissociation of the complex. If the complex does not dissociate during the chromatographic time scale, the complex is defined as an *inert species*. The free Cd ion and the Cd complex can be separated quantitatively as two independent peaks. If most of the complex dissociates during the chromatographic time scale, and only one peak appears in the chromatogram at the retention time of the free Cd ion, this complex is defined as a *labile species*. If a portion of the complex dissociates, the complex is defined as a *moderately labile species*. The adjusted retention time (t_R') of a target species represents reasonably well the time scale available for dissociation.

Moreover, the dissociation rate constant for the *moderately labile species* can be determined by comparing the determined complex percentage with that of its theoretical species distribution, if the free Cd ion and the complex involved in the equilibrium can be separated as two peaks. Otherwise, if they cannot be separated, and an "average" chromatographic behavior is observed, this means that one peak appears at a t_R between those of the free Cd ion and the complex. Then, the following relationship holds true [39,40]:

$$k = k_{Cd} x_{Cd} + k_{CdL} x_{CdL} \quad (5)$$

where k is the observed "average" retention factor, which is the weighted average of limiting retention factors of species coexisting in one peak and calculated based on the definition of $k = t_R'/t_0$. k_{Cd} and k_{CdL} represent the retention factors for the free Cd ion and CdL. x_{Cd} and x_{CdL} are the mole fractions of the free Cd ion and CdL, respectively. x_{Cd} reflects the labile degree for a given Cd species, showing the competition between L and the IPR for the free Cd ion and can be estimated using the following equation:

$$x_{Cd} = \frac{[Cd]}{[Cd]_{total}} \times 100\% = \frac{[Cd]}{[Cd] + [CdL] + [Cd\text{–}IPR]} \times 100\%$$
$$= \frac{1}{1 + K_{CdL}[L] + K_{Cd-IPR}[IPR]} \times 100\% \quad (6)$$

where [Cd] is the free Cd ion, which comes from the dissociation of CdL at time t_R' and $[Cd]_{total}$ is the total Cd concentration in the system. In general, CdL is negatively charged or neutral, and should not be retained on the IPR modified stationary phase of the column, that is, $k_{CdL} = 0$. Thus, Eq. (5) can be simplified as:

$$k = k_{Cd} x_{Cd} \quad (7)$$

Thus, x_{Cd} can also be obtained from Eq. (7), reflecting the molar fraction of dissociated CdL at t_R', and is thus defined as the lability of Cd species.

4. Results and discussion

4.1. Performance of IP-RP HPLC–ICP-MS system for the determination of Cd species

For the determination of Cd using IP-RP HPLC–ICP-MS, the linear range of Cd is from 5.0×10^{-8} to 5.0×10^{-5} mol L^{-1} with a linear regression coefficient of 0.9998. The relatively standard derivation was 3.0% for 5.0×10^{-8} mol L^{-1} Cd^{2+} solution ($n = 5$), and the detection limit (3σ) was calculated to be of 1.4×10^{-9} mol L^{-1}.

Ammonium acetate was used as an optimal eluent because its pH value can be easily adjusted from acidic to slightly basic without impairing the eluent strength, helping to minimize the possible shift in complexation equilibrium during the IP-RP HPLC process. The separation mechanism can be assigned by evaluating the dependence of retention factor k on the eluent concentration ($[NH_4^+]$). If cation exchange mechanism is predominant, a linear correlation should be found for a plot of $\log k$ vs. $\log[NH_4^+]$ [38]. In our study, the cation exchange behavior of ODS modified with SDS was investigated by repeatedly injecting Cd^{2+} solution while changing the ionic strength of eluent. As a result, a slope of 1.98 and linear regression coefficient of 0.9997 was obtained by log–log plot analysis. NH_4^+ has been found to be the only eluting cation and the retention of Cd^{2+} is controlled by cation-exchange mechanism on the SDS modified ODS column. Negatively charged complexes like Cd–EDTA were not retained on the column.

The free Cd and its complex separated by IP-RP HPLC lost their structural configuration during the processes of desolvation, vaporization, atomization, and ionization in the ICP, and yielded the same Cd signal on the mass spectrometer, which should be independent of the Cd species. To verify the non-species dependence of ICP-MS, a series of solution containing 5.0×10^{-6} mol L^{-1} Cd and 0.5, 1.0, 2.0, 5.0, 10, and 25×10^{-6} mol L^{-1} EDTA were prepared and analyzed by the IP-RP HPLC–ICP-MS system. Peak area for Cd–EDTA and Cd was obtained by area integration, RSD of the total area of Cd–EDTA and Cd was 2.1%, indicating that the sensitivity for Cd complex with EDTA was not significantly different from that of free Cd.

4.2. Dependence of the lability of a Cd species on its log K_{CdL}

The stability of the metal complex is an important factor that determines whether the complex remains intact or dissociates during the chromatographic process. In order to study the lability of Cd species during the separation process, Cd complexes with a broad range of equilibrium stability constants were chosen for this purpose. Theoretical calculation of Cd species distribution in the presence of DTPA, EDTA, HEDTA, PDCA, NTA, HIDA, HQS, and citrate indicated the fraction of positively charged Cd species is low

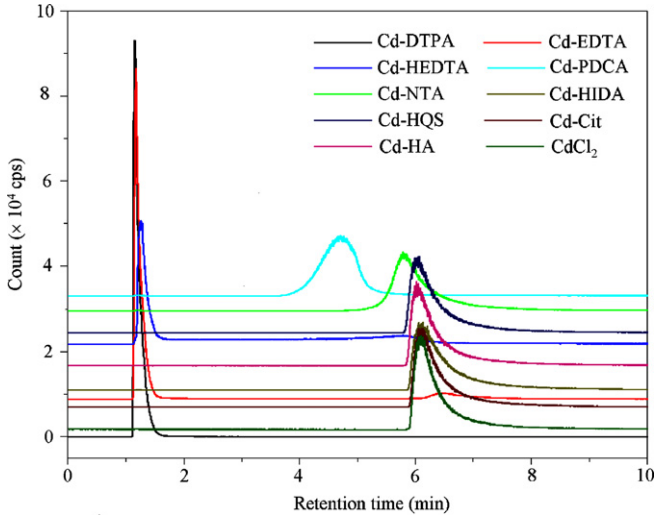

Fig. 1. Chromatograms of different Cd species. Cadmium concentration is 5.0×10^{-6} mol L^{-1}; ligand concentrations are 5.0×10^{-4} mol L^{-1} for citrate, HA, HQS, HIDA; and 5.0×10^{-5} mol L^{-1} for NTA, PDCA, HEDTA, EDTA and DTPA. Stationary phase, SDS modified ODS; mobile phase, mixture of 0.1 mol L^{-1} NH_4Ac–HAc (pH 6.0) at 0.7 mL min^{-1} under 25 °C.

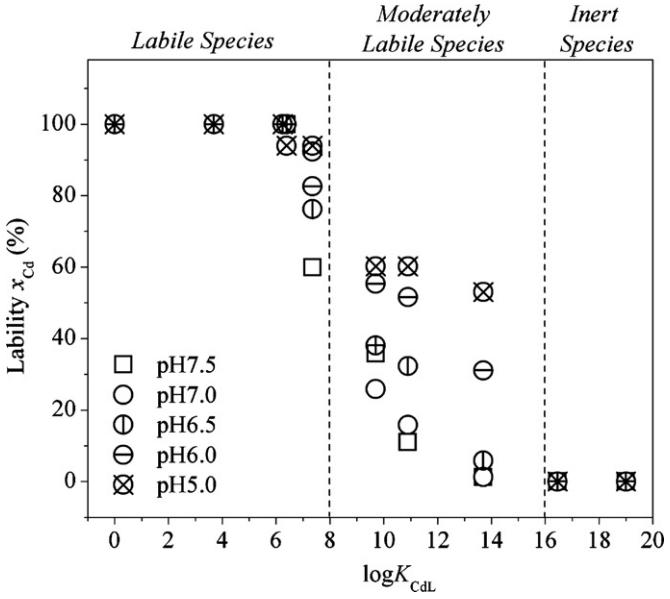

Fig. 2. Classification of the lability of Cd species using IP-RP HPLC–ICP-MS at different pHs from 5.0 to 7.5 on the SDS modified ODS stationary phase. The value of log K_{CdL} on the abscissa represent the model ligand of nitrate, citrate, HA, HQS, HIDA, NTA, PDCA, HEDTA, EDTA and DTPA (left to right). Mobile phase, a mixture of 0.1 mol L^{-1} NH_4Ac–HAc; flow rate, 0.7 mL min^{-1}; column temperature, 25 °C. Cadmium concentration is 5.0×10^{-6} mol L^{-1}, ligand concentrations are 5.0×10^{-4} mol L^{-1} except for EDTA and DTPA, which is 5.0×10^{-5} mol L^{-1}.

and the negatively charged Cd species are the dominant species. Only neutral Cd–HQS and Cd–HIDA are predominant in the solution. The retention behavior of Cd species on the cation exchange column depends mainly on their charge. However, when dissociation of the negatively charged Cd species occurs on the time scale of analysis, the retention behavior of this species will be different from their theoretical Cd species distribution. The classification of the lability of Cd species (x_{Cd}) was then dependent on the complex dissociation degree during the separation process and correlated to the retention factor k as defined in Eq. (7).

Typical chromatograms of different Cd species is shown in Fig. 1, the elution order is (Cd–DTPA, Cd–EDTA) > Cd–HEDTA > Cd–PDCA > Cd–NTA > (Cd–HIDA, Cd–HQS, Cd–HA, Cd–Cit and Cd^{2+}). The lability of Cd species displayed an inverse relationship with log K_{CdL} at different pHs as shown in Fig. 2. From the retention behavior of each Cd species, three categories were established: *inert species* ($x_{Cd} = 0$) with the log K_{CdL} bigger than 16, *moderately labile species* ($0 < x_{Cd} < 100\%$) with log K_{CdL} between 8 and 16, and *labile species* ($x_{Cd} = 100\%$) with the log K_{CdL} smaller than 8.

4.2.1. Inert species

Typical chromatograms of Cd–DTPA with different molar ratios of DTPA to Cd are shown in Fig. 3. Cd–DTPA and Cd–EDTA (log $K_{Cd-DTPA} = 19$ and log $K_{Cd-EDTA} = 16.42$) were eluted at hold time at all pH values studied, indicating that the negative charge of these species was not changed on the column. For Cd–DTPA and Cd–EDTA, free Cd ion and Cd–DTPA or Cd–EDTA (not shown, similar to Cd–DTPA) could be separated as two peaks. The peak area of Cd–DTPA or Cd–EDTA increased along with the increase in the total ligand concentration, while that of the free Cd ion decreased.

4.2.2. Moderately labile species

Chromatograms of Cd–HEDTA with different molar ratios of Cd to HEDTA are shown in Fig. 4a and considered as a typical *moderately labile species*. Cd–HEDTA (log $K_{Cd-HEDTA} = 13.7$) was eluted after Cd–EDTA. As HEDTA concentration increased, a platform appeared in the chromatogram due to incomplete dissociation of Cd–HEDTA on the column. For other types of *moderately labile species* like Cd–PDCA (see Fig. 4b) and Cd–NTA (not shown), when the ligand concentrations are 10 times over that of Cd, binary complex Cd–$PDCA_2^{2-}$ and Cd–NTA are predominant in the solution. However, negatively charged species was not found that individual species could not be separated and only one peak appeared in the chromatogram. The peak position is correlated with the ratio of Cd to PDCA or NTA. The retention factor demonstrated an "average" characteristic as described in Eq. (5) and reflected the partly dissociation of these species on the column.

4.2.3. Labile species

Other ligands, such as HIDA, HQS, Cit and HA, are known to form complexes with Cd with smaller stability constants. For Cd–HIDA ($K_{Cd-HIDA} = 10^{7.35}$), Cd–HQS ($K_{Cd-HQS} = 10^{6.4}$), Cd–Cit ($K_{Cd-Cit} = 10^{3.7}$, $K_{Cd-(Cit)2} = 10^{5.3}$), and Cd–HA ($K_{Cd-HA} = 10^{6.2}$) [41], although the lig-

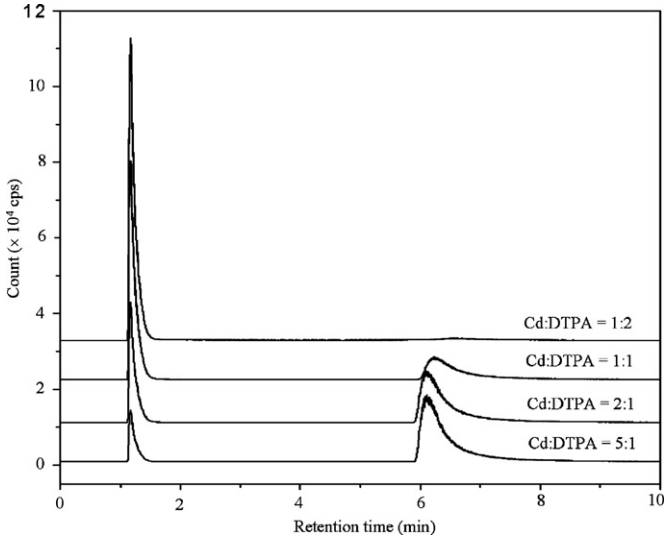

Fig. 3. Chromatograms obtained at various ratios of Cd to DTPA. Cadmium concentration is 5.0×10^{-6} mol L^{-1}; stationary phase, SDS modified ODS; mobile phase, a mixture of 0.1 mol L^{-1} NH_4Ac–HAc (pH 6.0) at 0.7 mL min^{-1} under 25 °C.

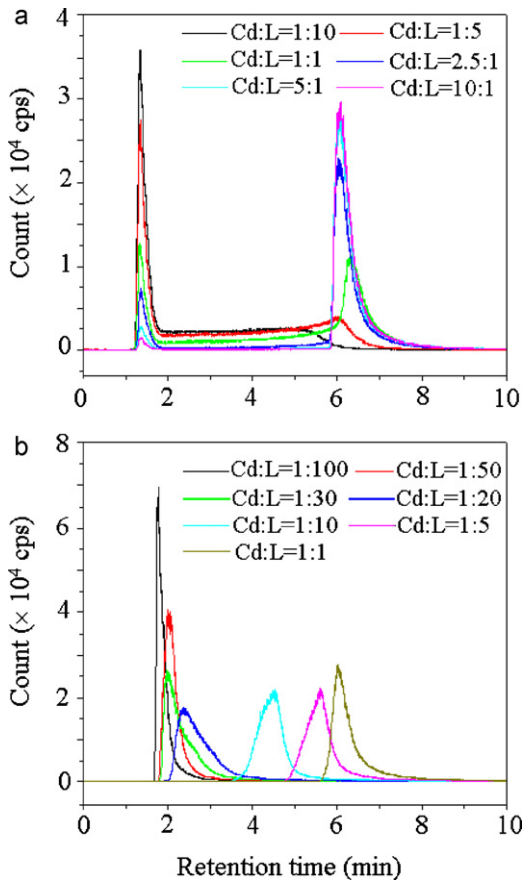

Fig. 4. Chromatograms obtained at (a) various ratios of Cd to HEDTA; (b) various ratios of Cd to PDCA. Cadmium concentration is 5.0×10^{-6} mol L^{-1}; stationary phase, SDS modified ODS; mobile phase, the mixture of 0.1 mol L^{-1} NH_4Ac–HAc (pH 6.0) at 0.7 mL min^{-1} under 25 °C.

ands concentration were 100 times over that of Cd^{2+} (except HA), more than 99.9% Cd existed as labile species after analysis using the IP-RP HPLC–ICP-MS (Fig. 1). Similar chromatographic behaviors of other *labile metallic species* were observed by Feng et al. [42] and in our previous studies [43–45]. HA does not form a stable species with Cd, which is abundant in the environment. The labile characteristic Cd–HA suggests its more active behavior than expected during the biosorption process.

4.3. Thermodynamic and kinetic characteristics of different Cd species on the column

4.3.1. Inert species

The proportions of free Cd ion and Cd–DTPA or Cd–EDTA when the concentrations of DTPA and EDTA increased from 5.0×10^{-7} to 1.0×10^{-5} mol L^{-1} were assessed based on the ratio of their corresponding peak areas. When the relative peak area of the Cd complex is compared with that of the theoretical species distribution, a little less Cd complex is detected than thermodynamically expected. For example, theoretical calculation indicated that equal mole concentration of EDTA with Cd (5.0×10^{-6} mol L^{-1}) should form 100% Cd–EDTA complex. Whereas, relative peak area of Cd–EDTA detected is only about 80%, implying that although most of the inert Cd species are stable during the separation process, a little part of Cd–DTPA and Cd–EDTA was activated in the IP-RP HPLC system. When an excess of the ligand was used (Cd:L = 1:2), relative peak areas of Cd–DTPA and Cd–EDTA reached a maximum value with about 96% Cd existing in the form of Cd–DTPA and/or Cd–EDTA. Thus, the complexation of inert species like Cd–DTPA and Cd–EDTA is very fast and the excess ligand prevents Cd from undergoing other side reactions such as binding to the IPR on the column.

Through the so-called log–log plot analysis of log([Cd–DTPA]/[Cd]) against log[DTPA] and log([Cd–EDTA]/[Cd]) against log[EDTA], the conditional stability constants of Cd–DTPA and Cd–EDTA were obtained. The equation for Cd–DTPA was $y = 1.38x + 8.51$ with a correlation coefficient r^2 of 0.9838 and for Cd–EDTA was $y = 1.12x + 6.61$ with r^2 of 0.9460. The conditional stability constants of Cd–DTPA and Cd–EDTA were estimated to be $10^{8.51}$ and $10^{6.61}$, respectively. Taking into consideration that the side reaction of Cd complex with acetate ion (Ac^-) in the mobile phase ($\alpha_{(Cd)} = 5.3$) and the acidic effect of DTPA ($\alpha_{DTPA(H)} = 10^{7.1}$) and EDTA ($\alpha_{EDTA(H)} = 10^{4.7}$) at pH 6.0, the conditional stability constants of Cd–DTPA and Cd–EDTA are $10^{11.18}$ and $10^{11.08}$. This suggests the reaction coefficients of Cd binding to the IPR on the column are $10^{2.6}$ and $10^{4.5}$ in DTPA and EDTA.

4.3.2. Moderately labile species

As *moderately labile species*, the species distribution of free Cd ion and Cd–HEDTA are significantly different from that of theoretical calculations. For example, 5×10^{-6} mol L^{-1} HEDTA should theoretically cause 99.8% of Cd complexation, while the relative peak area of Cd–HEDTA found is only 32%. When the concentration of HEDTA exceeded 10 times that of Cd^{2+}, the peak belonging to Cd–HEDTA became dominant. Compared with the theoretical stability constant of Cd–HEDTA ($10^{13.7}$) in solution, the conditional stability constant of Cd–HEDTA determined by the IP-RP HPLC–ICP-MS decreased to $10^{7.23}$ (linear equation for Cd–HEDTA was $y = 1.42x + 7.23$ with r^2 of 0.9936). Using Eq. (4), the dissociation rate constant k_d of Cd–HEDTA was determined to be 8.7×10^{-3} s^{-1}. These values imply that *moderately labile species* would become more labile in complicated environmental systems. For other types of typical *moderately labile species*, the retention factor of Cd–PDCA and Cd–NTA demonstrated an "average" characteristic as described in Eq. (5). When the ligand concentration increased from 5×10^{-6} to 5×10^{-4} mol L^{-1}, the retention time of Cd–PDCA decreased from 6.0 min to 1.83 min. The conditional stability constants of Cd–PDCA and Cd–NTA can be obtained from Broul's equation ($1/k = A(B + K_{CdL}[L])$ [38], where k is the observed retention factor, A is the constant from the combined column parameter, selective coefficient, and a constant concentration of elution cation; and B denotes the degree of side reaction of Cd with Ac^- in the mobile phase, which is 5.3 in this study. By plotting $1/k$ against [L] from 1×10^{-5} to 1×10^{-3} mol L^{-1}, the linear equation for Cd–PDCA was $y = 910x + 0.35$ with r^2 of 0.9533, and for Cd–NTA $y = 528x + 0.3$ with r^2 of 0.9764. The conditional stability constants were estimated to be $10^{4.1}$ for Cd–PDCA and $10^{4.0}$ for Cd–NTA, which decreased significantly compared with their theoretical thermodynamic stability constants ($10^{10.9}$ for Cd–$PDCA_2$ and $10^{9.8}$ for Cd–NTA). On the other hand, the dissociation rate constant k_d of the species can be determined from the slope of the plot of $\ln([CdL]_0/[CdL]_t)$ against the adjusted retention time t_R' according to Eq. (4). The equations for Cd–PDCA and Cd–NTA were $y = 8.4 \times 10^{-3}x - 0.5$ with r^2 of 0.9471 and $y = 1.0 \times 10^{-2}x - 0.5$ with r^2 of 0.9293, respectively. The k_d of Cd–PDCA and Cd–NTA were thus estimated to be 8.4×10^{-3} and 1.0×10^{-2} s^{-1}, respectively.

4.4. Cd bioaccumulation of P. tricornutum in the presence of Cd species with different labilities

Cd bioaccumulation in *P. tricornutum*, which is a typical unicellular marine diatom, was evaluated in order to evaluate the lability-dependent bioavailability of Cd species. *P. tricornutum* has high endurance of Cd (96 h Cd EC50 value for *P. tricornutum* is as high as 22.39 mg L^{-1}) [46] and capable to synthesis PCs in the cellular under Cd exposure [36,47]. Short time exposure (40 min)

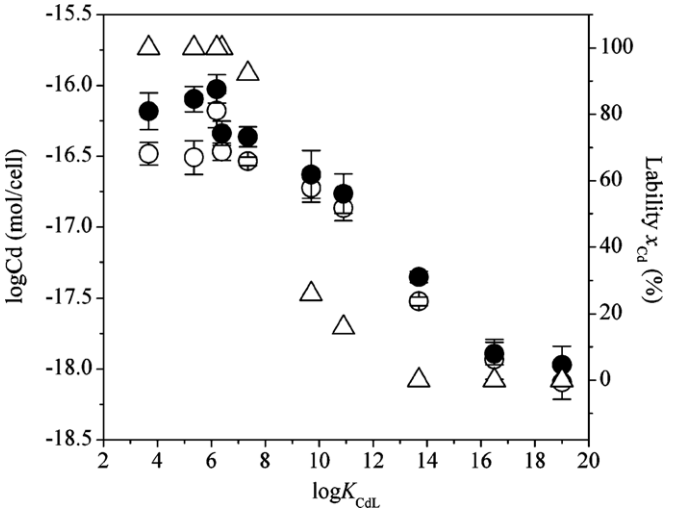

Fig. 5. Surface-bound Cd (filled circle) and internalized Cd (hollow circle) content in *P. tricornutum* (left *Y* axis) and species lability x_{Cd} (hollow triangle) (right *Y* axis). The value of $\log K_{CdL}$ on the abscissa represents the logarithm of stability constants of Cd species with Cit, HA, HQS, HIDA, NTA, PDCA, HEDTA and EDTA (left to right). Cadmium concentration is 4.5×10^{-5} mol L^{-1}, ligand concentrations are 4.5×10^{-4} mol L^{-1} except for HA, which is 5 mg L^{-1}; same concentration of $CdCl_2$ solution was used in the control group. Error bars represent the standard deviation from 3 experiments.

in solution containing 4.5×10^{-5} mol L^{-1} cadmium did not cause acute toxicity to *P. tricornutum*. Results of surface-bound and internalized Cd content when *P. tricornutum* is exposed to different Cd species with different labilities (x_{Cd}) are illustrated in Fig. 5. Surface-bound Cd concentration is higher than that of internalized Cd in general, and both surface-bound and internalized Cd increased along with the increase in the lability of Cd species or increased free Cd ion concentration when the total amount of Cd in the culture solution is the same (4.5×10^{-5} mol L^{-1}). Thus, Cd bioaccumulation by *P. tricornutum* can be predicted by the lability of Cd species. This gives evidence that when trace metal speciation cannot be rigorously controlled and measured, the estimate of the lability of metal species using the proposed IP-RP HPLC–ICP-MS method is helpful in predicting the bioavailability and subsequent toxicity of different metal species.

5. Conclusion

The established IP-RP HPLC–ICP-MS system enables the classification of the noncovalent-bonded Cd species into *inert*, *moderately labile*, and *labile species* based on the corresponding chromatographic behavior. This method is applied in evaluating the lability-dependent bioavailability of Cd species to *P. tricornutum*. The uptake of Cd by *P. tricornutum* increased along with the increase in the lability of Cd species. Considering the dynamic distribution process of Cd species between the stationary and mobile phases are similar to those between heterogeneous interfaces in biological and environmental systems, such a methodology is expected to be useful for evaluating and forecasting the bioavailability and/or toxicity as well as the mobility of noncovalent-bonded metal species.

Acknowledgements

This study was financially supported by the National Natural Sciences Foundation of China (21035006, 20775062 and 20175019) and the National Basic Research Program of China (2009CB41605). Ocean Environment and Fishery Source Monitor Center of Fujian Province is gratefully appreciated for providing the instruments.

References

[1] P.G.C. Campbell, in: A. Tessier, D.R. Turner (Eds.), Metal Speciation and Bioavailability in Aquatic Systems, John Wiley, New York, 1995, pp. 45–102.
[2] G.E. Batley, S.C. Apte, J.L. Stauber, Aust. J. Chem. 57 (2004) 903–919.
[3] R. Biesuz, G. Alberti, G. D'Agostino, E. Magi, M. Pesavento, Mar. Chem. 101 (2006) 180–189.
[4] J. Buffle, M.L. Tercier-Waeber, Trends Anal. Chem. 24 (2005) 172–191.
[5] S. Jansen, R. Blust, H.P. Van Leeuwen, Environ. Sci. Technol. 36 (2002) 2164–2170.
[6] P. Figura, B. McDuffie, Anal. Chem. 52 (1980) 1433–1439.
[7] H.P. Van Leeuwen, R.M. Town, J. Buffle, R.F.M.J. Cleven, W. Davison, J. Puy, W.H. Van Riemsdijk, L. Sigg, Environ. Sci. Technol. 39 (2005) 8545–8556.
[8] L. Sigg, F. Black, J. Buffle, J. Cao, R. Cleven, W. Davison, J. Galceran, P. Gunkel, E. Kalis, D. Kistler, M. Martin, S. Noel, Y. Nur, N. Odzak, J. Puy, W. Van Riemsdijk, E. Temminghoff, M.L. Tercier-Waeber, S. Toepperwien, R.M. Town, E. Unsworth, K.W. Warnken, L.P. Weng, H.B. Xue, H. Zhang, Environ. Sci. Technol. 40 (2006) 1934–1941.
[9] H.P. Van Leeuwen, Environ. Sci. Technol. 33 (1999) 3743–3748.
[10] L.P. Weng, W.H. Van Riemsdijk, E.J.M. Temminghoff, Anal. Chem. 77 (2005) 2852–2861.
[11] C. Belmont-Hébert, M.-L. Tercier, J. Buffle, G.C. Fiaccabrino, M. Koudelka-Hep, Anal. Chem. 70 (1998) 2949–2956.
[12] S. Scally, W. Davison, H. Zhang, Environ. Sci. Technol. 37 (2003) 1379–1384.
[13] M.-H. Tusseau-Vuillemin, R. Gilbin, M.A. Taillefert, Environ. Sci. Technol. 37 (2003) 1645–1652.
[14] R. Lobinski, J. Szpunar, Hyphenated Techniques in Speciation Analysis (seria RSC Chromatography Monographs), RSC, Cambridge, 2003, pp. 53–64.
[15] K.L. Ackley, J.A. Caruso, in: R. Cornelis, J. Caruso, H. Crews (Eds.), Handbook of Elemental Speciation: Techniques and Methodology, John Wiley and Sons, Chichester, 2003, pp. 147–162.
[16] J. Szpunar, Analyst 125 (2000) 963–988.
[17] A. Prange, D. Schaumloffel, Anal. Bioanal. Chem. 373 (2002) 441–453.
[18] M.J. Shaw, P.R. Haddad, Environ. Int. 30 (2004) 403–431.
[19] M. Montes-Bayon, K. DeNicola, J.A. Caruso, J. Chromatogr. A 1000 (2003) 457–476.
[20] Y. Tian, M.L. Chen, X.M. Chen, J.H. Wang, J. Anal. Atom. Spectrom. 25 (2010) 48–54.
[21] F. Zhang, B. Hu, J. Mass. Spectrom. 45 (2010) 205–214.
[22] J.E. Sonke, V.J.M. Salters, Analyst 129 (2004) 731–738.
[23] G. Borrmann, A. Seubert, Anal. Chim. Acta 386 (1999) 77–88.
[24] K. Tsunoda, T. Umemura, K. Ohshima, S. Aizawa, E. Yoshimura, K. Satake, Water Air Soil Pollut. 130 (2001) 1589–1594.
[25] K.C. Bowles, S.C. Apte, G.E. Batley, L.T. Hales, N.J. Rogers, Anal. Chim. Acta 558 (2006) 237–245.
[26] R.N. Collins, J. Chromatogr. A 1059 (2004) 1–12.
[27] P.M. Bertsch, M.A. Anderson, Anal. Chem. 61 (1989) 535–539.
[28] A. Raab, A.A. Meharg, M. Jaspars, D.R. Genney, J. Feldmann, J. Anal. Atom. Spectrom. 19 (2004) 183–190.
[29] D. Kratochvil, B. Volesky, Trends Biotechnol. 16 (1998) 291–300.
[30] J. Van Doornmalen, J.T. Van Elteren, J.J.M. De Goeij, Anal. Chem. 72 (2000) 3043–3049.
[31] R.M. Smith, A.E. Martell, R.J. Motekaitis, NIST Critical Selected Stability Constants of Metal Complexes Database, Version 6.0, US Department of Commerce, National Institute of Standards and Technology Standard Reference Data Program, Gaithersburg, MD, 1999.
[32] R.M. Cassidy, S. Elchuk, Anal. Chem. 54 (1982) 1558–1563.
[33] R.R.L. Guillard, in: W.L. Smith, M.H. Chanley (Eds.), Culture of Marine Invertebrate Animals, Plenum Publishing Corporation, New York, 1975, pp. 29–60.
[34] F.M.M. Morel, J.G. Reuter, J. Phycol. 15 (1979) 135–141.
[35] C.S. Hassler, V.I. Slaveykova, K.J. Wilkinson, Limnol. Oceanogr. Methods 2 (2004) 237–247.
[36] D.F. Si, L.M. Yang, H. Yan, Q.Q. Wang, Sci. China Ser. B: Chem. 52 (2009) 2373–2380.
[37] M. Xu, G.F. Deng, S.S. Liu, S. Chen, D. Cui, L.M. Yang, Q.Q. Wang, Metallomics 2 (2010) 469–473.
[38] P. Janoš, M. Broul, Fresenius J. Anal. Chem. 34 (1992) 545–548.
[39] P. Janoš, J. Chromatogr. A 1037 (2004) 15–28.
[40] D. Karcher, I.S. Krull, in: I.S. Krull (Ed.), Trace Metal Analysis and Speciation (Journal of Chromatography Library 47), Elsevier, Amsterdam, 1991, pp. 123–166.
[41] G. Abate, J.C. Masini, Org. Geochem. 33 (2002) 1171–1182.
[42] Y.Q. Feng, M. Shibukawa, K. Oguma, Chromatographia 41 (1995) 532–538.
[43] Q.Q. Wang, J.L. Liu, B.L. Huang, L.M. Yang, X.M. Guo, X.R. Wang, Anal. Sci. 16 (2000) 241–244.
[44] Q.Q. Wang, B.L. Huang, Z.W. Guan, L.M. Yang, B. Li, Fresenius J. Anal. Chem. 370 (2001) 1041–1047.
[45] B. Li, Q.Q. Wang, H. Yan, L.M. Yang, B.L. Huang, Anal. Bioanal. Chem. 376 (2003) 923–927.
[46] E. Torres, A. Cid, C. Herrero, J. Abalde, Bioresour. Technol. 63 (1998) 213–220.
[47] S.K. Kawakami, M. Gledhill, E.P. Achterberg, BioMetals 19 (2006) 51–60.