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Determination of strong binding chelators and their metal complexes by anion-exchange chromatography and inductively coupled plasma mass spectrometry

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Abstract

Based on the negative charge of polycarboxylic chelators, an anion-exchange separation has been developed that is compatible with sensitive metal detection by ICP-MS. A low capacity hydrophilic polymer (AS11) was used as the anion exchanger and ammonium nitrate as the eluent. The new procedure provided high selectivity in the isocratic mode as well as a large separation window and high separation efficiency in the gradient mode. This was demonstrated for different types of chelators and their metal complexes. The aminopolycarboxylates NTA, EDTA, CDTA, DTPA, EDDS and for the EDTA derivatives HEDTA, ED3A and EDTMP, the phosphonic acid analogue of EDTA were tested. Their retention times generally depended on the charge, which was lower in 1:1 metal chelator complexes. Evaluation of the separation mechanism demonstrated that they were all separated predominantly by an anion-exchange mechanism with only a minor contribution from hydrophobic attraction. The method is useful for species identification and for predicting the charge of unknown analogous species from retention times. A gradient separation procedure achieved on-column preconcentration and matrix removal for the interference-free detection of metal chelates down to low nanomolar concentration in samples from various fields of environmental research.

Keywords: Inductively coupled plasma mass spectrometry; Chelators; Metal complexes; Polycarboxylic acids

1. Introduction

The traditional use of chelators in metal masking and prevention of precipitation in various processes has been extended to applications such as the chelator-enhanced trace element fertilisation of crops [1,2] and animal nutrition, phytoremediation [3,4] and groundwater tracers [5]. The central role of aminopolycarboxylic acids in such processes and many aspects of their occurrence and degradation in the environment have recently been discussed in an excellent review [6]. Their widespread deployment in production and consumer goods has generated broad interest in separation methods for the determination of the total chelator concentration as well as for metal speciation. Concerns over the environmental impact have arisen from the increasing concentrations in receiving waters (rivers [7–9], groundwaters [10]) of chelators of limited degradability (e.g. EDTA [11,12]) or slow elimination rate (e.g. phosphonates [13]). Procedures have been established to determine the total chelator concentrations for such investigations. To obtain, for example, the total EDTA concentrations, all metal com-

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plexes have to be transformed chemically into a single readily UV-detectable Fe(III) complex [14–20]. Hence, these methods destroy the complexes and are not suitable for speciation studies. However, ecotoxicological and metal mobility investigations [21] require the direct observation of several metals bound to different chelates, which is the only way to obtain a more accurate picture, because non-equilibrium conditions [22–24] and species exchange and dynamics can be observed directly. Methods compatible with this requirement have been described. The potential of capillary electrophoresis (CE) was evaluated [25–27] by separation of metal EDTA and NTA complexes. Detection by UV absorption and laser-induced fluorescence [28] resulted in detection limits of 2–500 and 0.01–0.1 μ*M*, respectively. In the case of negatively charged metal chelates, anion-exchange chromatography proved to be a successful separation method. Carbonate eluents at pH >9 in combination with various columns were reported to elute these complexes, which were detected by conductivity [29,30] and UV–vis [31,32]. Suppressing the eluent improved the signal-to-noise ratio in ESI-MS determination [33] and allowed the detection of nanomolar concentrations. However, carbonate elutes anionic metal complexes together with sample ions NO_3^- and SO_4^{2-} at a pH where, for example, Al-, Fe- and Zn-chelates are not stable. The pH cannot be adjusted to a value below 9 without substantial loss in eluent strength, which is proportional to the increase of the weak eluent anion HCO_3^-.

This article reports the development of a new and extended application of anion-exchange chromatography to metal speciation covering the range of natural water (pH 6–8) which has broad selectivity. Coupling to ICP-MS was used for element identification and low detection limits for heavy metals [34]. Metal complexes of diverse environmentally relevant chelators were investigated. Their separation behaviour is described and method performance data discussed. The method was applied [35] to various fields of environmental research.

2. Experimental

2.1. Chemicals

Nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid disodium salt (EDTA), $ZnEDTANa_2$, *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid (HED3A), cyclohexane-1,2-diaminetetraacetic acid (CDTA), citric acid and di-ethylenetriaminepentaacetic acid (DTPA) were purchased from Fluka or Merck in the highest purity available. Ethylenediaminetriacetic acid (ED3A) and ethylenediaminedisuccinate (EDDS) were a kind gift from M. Bucheli-Witschel and T. Egli (EAWAG). Ethylenediaminetetramethylenephosphonic acid (EDTMP) was obtained as Dequest 2041 (Monsanto) from H. Felber (EMPA, St. Gallen).

Metal ligand (1:1 molar, except NTA and citrate—1:2) stock solutions (0.1 mmol/L) were prepared by adding the ligand solution (1 mmol/L) to the metal solution (diluted from a 1000 mg/L AAS standard, J.T. Baker) followed by neutralisation (NH_4OH). The total metal content was checked at intervals against a certified multi-element standard (Merck VI). Fe-chelates were stored in the dark. If not otherwise stated, the metal complex concentrations typically injected were 1 μmol/L. They were prepared daily from stock solutions.

Concentrations were calculated from peak integrals compared to linear calibration curves obtained from standards in purified water or added to the sample.

2.2. Chromatography

An all-PEEK system was used consisting either of a Bischoff 2200 isocratic pump (analytical size pump head) or a GP40 (Dionex) microbore gradient pump, a Rheodyne (9126) injection valve (270 μL loop) and a low capacity (0.011 mequiv.) microbore AS11 column (250×2 mm, Dionex) with hydrophilic quaternary alkanol ammonium as anion-exchange sites [36]. The eluent was prepared from conc. HNO_3 and ammonia (supra pure, Merck) in He-degassed purified water (18 MΩ). Eluents were kept under an over pressure of He to maintain a low carbon background. The eluent pH was adjusted with NH_4OH–HNO_3 and checked several times during chromatographic work at the pump head drain valve. Gradients were linear mixtures (0–8 min) of water and 200 m*M* NH_4NO_3 solution. The eluent flow (440 μL/min) was chosen for fast separation and the highest mass transfer by the nebulizer. Metals were removed on line from the eluents by passage through

a column (50×4 mm) filled with a precleaned strong cation exchanger (AG 50W-X8, Bio-Rad). The stability and reproducibility of the flow-rate, the evolving gradient and the nebulisation was observed at m/z 30 from NO^+ formed by disproportion of NH_4NO_3, which clearly indicated t_0 by the water dip. Sample preconcentration was achieved either by the sample loop used or, for larger volumes (1–5 mL), by injection on AG11 (50×4 mm, Dionex) in the loop position. Metal deposits from complexes of lower stability ($\log K < 9$) [13] were mobilised and controlled by injections of free chelator, as recommended by Hering [37] and Szpunar [38] for size-exclusion chromatography. Only free chelator was able to mobilise metal from the column. Calcium and heavy metal EDTA complexes did not.

2.3. Coupling and ICP-MS measurements

The column was connected to a micro concentric nebulizer (MCN-100 M2, CETAC) mounted on a Scott-type Ryton double-pass spray chamber. The ICP-MS (ELAN 5000, Perkin-Elmer–Sciex) operating conditions were as follows: RF power, 1100 W; Ar gas flow (L/min), plasma (15), auxiliary (1), nebulizer (0.8–1.0).

For fast sequential detection dwell times (25–100 ms), the number of replicates and the number of detected isotopes (m/z values) were selected according to the time needed to elute components and to detect them by at least 10 points/peak. Data were acquired in the Graphic mode. Single mass chromatograms and peak integrals were obtained from Chromafile MSplus software (LabControl). Element isotopes were detected at the m/z values (Me:m/z(abundance)) of minimal interference: C, 12 (98.9%); Fe, 57 (2.2%); Co, 59 (100%); Ni, 60 (26.1%); Cu, 65 (30.8%); Zn, 66 (27.9%); Cd, 111 (12.8%); and Pb, 208 (52.4%). Chromatography was tested for coelution of species containing elements that interfere with element detection of the species under investigation. The absence of, for example, Na during the elution of Cu species and the absence of Sn during the elution of Cd species allows the detection of isotopes of higher abundance [Cu, 63 (69.2%); Cd, 114 (28.7%)] for a higher detection sensitivity. During the acquisition of high signals (e.g. m/z 12 or 30) the detector had to be desensitised (omni range 10–20) because detector overflow in one chromatogram caused data cutoff in all other chromatograms of the same run while reading the data with Chromfile.

3. Results and discussion

3.1. Eluent and column selection

Polycarboxylic agents and their complexes are polyvalent anions. As such they are strongly retained by a polymeric anion exchanger. This was observed for a reversed-phase column permanently coated with a lipophilic ammonium compound. Phosphate and sulfate are required to elute heavy metal EDTA complexes [39]. Such salts are not compatible with ICP-MS since they cause undesirable deposits and interference. Ammonium nitrate was chosen as the optimal eluent because the anion has a high affinity for the anion exchanger [40] and the lowest metal complex stability constants. Both NH_4^+ and NO_3^- decompose completely into volatile gaseous components at high temperature. The plasma tolerated eluent concentrations well above 0.15 mol/L NH_4NO_3; it was never extinguished and no deposits could be observed. No sensitivity loss for ^7Li, ^{65}Cu and ^{103}Rh (10 μg/L solution) in 0.15 mol/L NH_4NO_3 was observed compared to the same concentrations prepared in 0.14 mol/L HNO_3, indicating no difference in space charge effects [41]. This broad eluent tolerance by the plasma allowed gradients to be applied that opened up the analytical window in an unprecedented manner (see below).

The pH of the eluent can be adjusted to a broad range down to acidic pH, if necessary, without compromising on eluent strength. Most metal complexes, however, are destabilised under non-neutral pH conditions ($6 > pH > 8$). An increasing amount of NH_3 at higher values ($pH > 8$) may help to destabilise some complexes. At acidic pH, the loss of buffering capacity of the eluent requires the sample and eluent pH to be identical.

3.2. Isocratic characterisation of free chelators and their metal complexes

The selected chelators (see Table 1) are all artificial, but one is of biogenic (EDDS) origin, and represent diverse aspects of the complexation

chemistry as well as environmental concern [6]. The formal anionic charge of the species varied between −1 and −6. The metal complexes represent a broad range of equilibrium stability constants [42] and they play an important role in environmental metal speciation [22–24]. The selected metals react at different rates [43], for example Ni is representative of a slow, and Pb of a fast, reacting metal.

To test the selectivity of the anion-exchange procedure, isocratic elution was investigated for the uncomplexed ligands given in Table 1. For the chromatographic conditions applied (pH 7 and 8), equilibrium calculations (VisualMINTEQ [44]) and equilibrium constant considerations [42] showed that citrate was almost completely deprotonated, whereas the N-containing chelators were protonated, bearing the formal negative charge numbers given in Table 1. From the peak detected at m/z 12, retention (formerly: capacity) factors (k') were calculated and are presented in Fig. 1. All chelators showed an almost exponential increase of k' with decreasing eluent concentration. Such an increase in k' is typical for multiply charged analytes and was accompanied by peak broadening, so that peaks with $k' > 6–8$ were difficult to integrate. Generally, the higher the net charge of the free chelator, the higher the eluent concentration required to elute the compound as an integrable peak. Similar k' values were found for

Table 1
Species characteristics and measured retention times (t_R) in isocratic elution

Ligand and complex	Species charge	Species distribution[a] (%)		t_R (min) (eluent, mM)	Log K range[b]
		pH 7	pH 8		
NTA	−3	0.1	1.4	–	
NTAH	−2	99.9	98.6	4.0 (20)	
MeNTA, $Me(NTA)_2$	−1 to −4	99–100	89–100	4.0–5.5 (20)	10–16
HED3AH	−2			3.0 (20)	
MeHED3A	−1			2.0 (20)	13–20
ED3AH	−2			3.0 (20)	
MeED3A	−1			2.0 (20)	
EDDSH	−3			3.0 (50)	
MeEDDS	−2			2.0 (50)	11–15
EDTAH	−3	93.4	99.3	3.5 (50)	
$EDTAH_2$	−2	6.6	0.7	–	
MeEDTA	−2	99–100	100	2.0 (50)	16–25
CDTAH	−3			4.0 (50)	
MeCDTA	−2			2.4 (50)	17–30
DTPAH	−4	2.5	20	–	
$DTPAH_2$	−3	97.5	80	–	
MeDTPA	−3				18–28
Citr	−3	92.7	99.2	4.0 (70)	
CitrH	−2	7.3	0.8	–	
MeCitr, $Me(Citr)_2$	−1 to −4			–	4–6
$EDTMPH_{2.6}$	−5.4			4.7 (130)	
MeEDTMPH	−5.0 to −5.5			3.5–4.0 (130)	15–23
NH_4^+		99.5	95.6	–	
NH_3		0.5	4.4	–	

[a] Species distribution was calculated [44] for 50 mmol/L NH_4NO_3, free chelator 0.1 mmol/L and metal species 1 μmol/L.
[b] Log K values for Me = Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) were taken from Ref. [42] (I = 0.1, 25 °C).

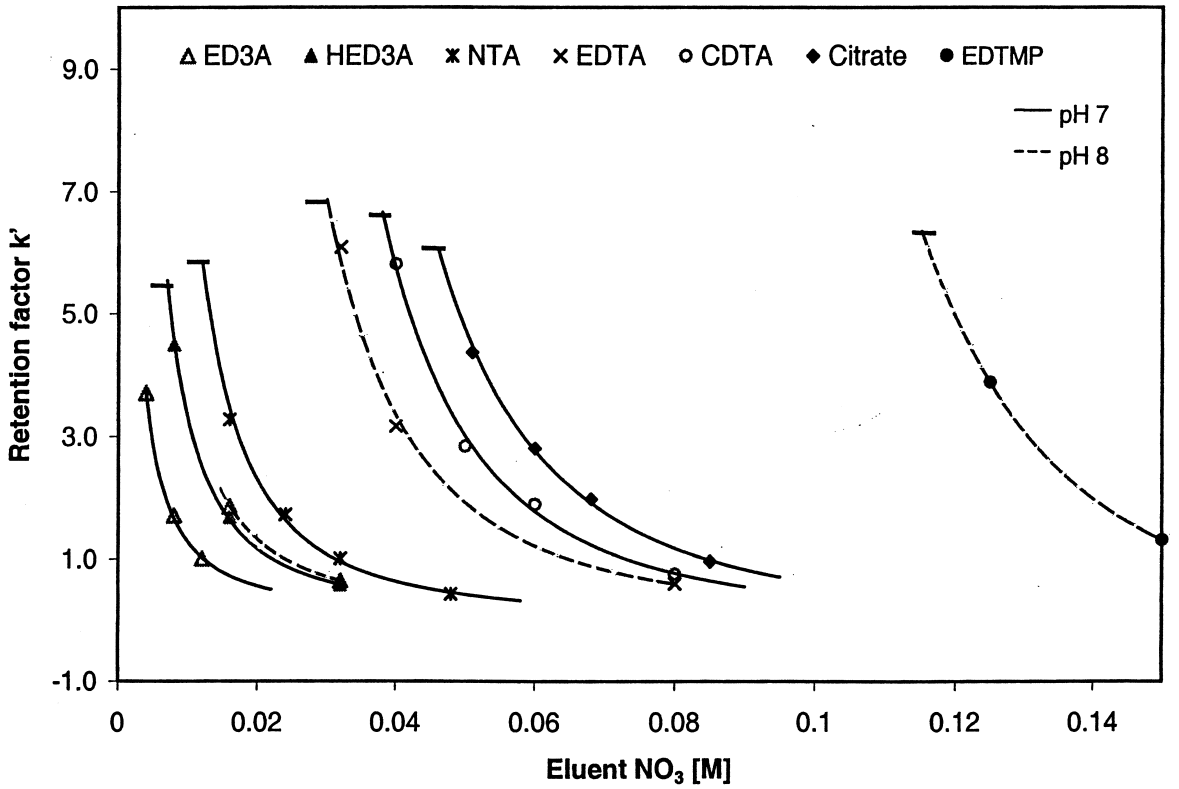

Fig. 1. Anion-exchange behaviour of free chelators characterised by retention factors k' for $(NTAH)^{2-}$, $(ED3AH)^{2-}$, $(HED3AH)^{2-}$, $(EDTAH)^{3-}$, $(CDTAH)^{3-}$, $Citrate^{3-}$ and $(EDTMPH_{2.6})^{5.4-}$. Injections of 50–100 μmol/L solutions (except NTA: 200 μmol/L) and elution at different isocratic eluent concentrations. Lines represent a fit of the data points to a quadratic function. Horizontal dashes mark a k' value with a too large peak broadening for integration.

species of equal formal charge number. The retention factors of these polycarboxylates did not vary significantly between eluent pH values of 7 and 8, except for ED3A and EDDS, which contain at least one secondary nitrogen which has a lower pK_a than the tertiary one in HED3A and EDTA. $Citrate^{3-}$ and $(EDTAH)^{3-}$, with the same formal charge, are separated from each other. This can be explained by the charge density. Since EDTA is the larger molecule, it has a smaller charge density and requires a lower eluent concentration. However, the same applies for CDTA, which is larger than EDTA (but identical pK_{a3} [42]), and should therefore elute before EDTA. The fact that the latter elutes before CDTA can be explained by a special feature of this molecule (see Section 3.3).

In 1:1 complexes of these ligands (L) with heavy metals (Me), the negative charge is reduced compared to the free ligand. Therefore, a clear shift towards shorter retention times should be observed. As can be seen from the data in Fig. 2 this was observed for $(EDTAH)^{3-}$ and $(Me(II)EDTA)^{2-}$, which are known to form exclusively 1:1 MeL complexes [45–49]. Free $(EDTAH)^{3-}$ eluted later than all the $(Me(II)EDTA)^{2-}$ species. The latter were clearly separated in complexes with a larger metal ionic radius (Cd^{2+}, Pb^{2+}) and those EDTA complexes of smaller metal ionic radius (Co^{2+}, Ni^{2+}, Cu^{2+}). A similar elution sequence was observed with other eluents on a silica-based [29,30], and on a medium hydrophobic polystyrene [50] anion-exchange, column. This sequence can again be explained by the lower charge density of $(Me(II)EDTA)^{2-}$ formed by larger metal ions. Lower eluent concentrations provided higher selectivity and separated all the investigated metal complexes in a characteristic sequence.

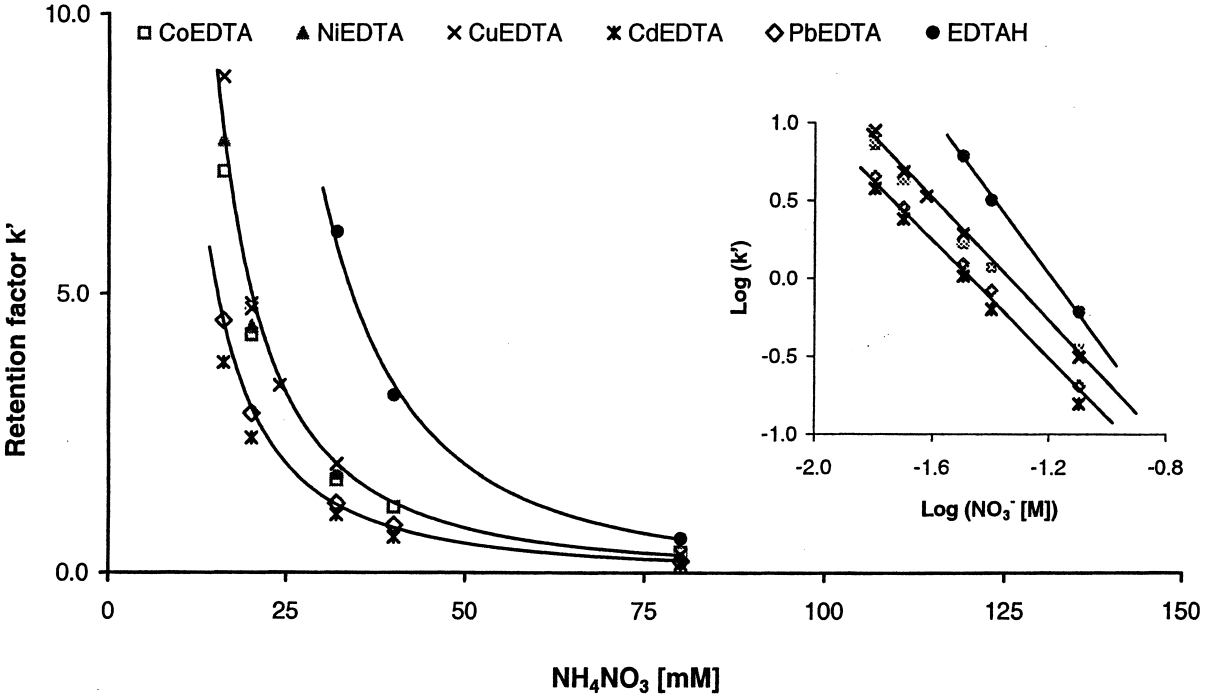

Fig. 2. Retention factors k' for $(EDTAH)^{3-}$ and its metal complexes $MeEDTA^{2-}$ ($Me = Co^{2+}$, Ni^{2+}, Cu^{2+}, Cd^{2+}, and Pb^{2+}). Conditions as in Fig. 1, metal complexes injected as 1 μmol/L solutions. Insert: log–log plot of the data.

Therefore, the presence of several metal complexes from the same chelator can provide a separation pattern that can identify the chelator. This is the unique advantage of element-specific detection: the use of such a metal species separation pattern for ligand identification.

EDTA structure analogues such as HED3A, ED3A, CDTA and EDTMP, which also form predominantly 1:1 complexes [42], showed the same relative elution sequence as found for EDTA between the free ligand and its metal complexes. Fig. 3 shows the retention factors of the free ligands together with selected metal complexes. DTPA was found to behave differently since it contains two protons in the non-metallic species $(DTPAH_2)^{3-}$ which are replaced in metal complexes. Hence, Me^{2+} form $(Me(II)DTPA)^{3-}$ species with the same formal negative charge, which explains the almost identical retention times obtained for these species.

The other ligands, without the ethylenediamine structural unit, such as citrate and NTA (Fig. 4), differ considerably with respect to coordination and stability of the complexes. They are known to form $Me(II)L_2$ and $(Me(II)L)_2$ as well as Me(II)L, due to the smaller size and less coordinating ligand atoms. Accordingly, the elution sequence for NTA and its metal complexes (Fig. 4) could possibly be explained by a $(Me(II)(HNTA)_2)^{2-}$ species, where partial deprotonation increases the anionic charge density compared to $NTAH^{2-}$. For $(Pb(II)(HNTA)_2)^{2-}$, the charge density would be reduced because of the larger size of Pb^{2+} compared to Ni^{2+} and Cu^{2+}.

Metal binding is in competition between the chelator and the column. Therefore, the stability of MeL is an important prerequisite that can decide whether the complex remains intact or is decomposed during chromatography, the metal being deposited on the column. Table 2 lists the metal fractions deposited from complexes of different stability. It illustrates the required complex stability ($\log K > 10$) and allows an estimation of metal binding to the column ($\log K = 8$–9). Citrate forms much less stable complexes, so that metals from

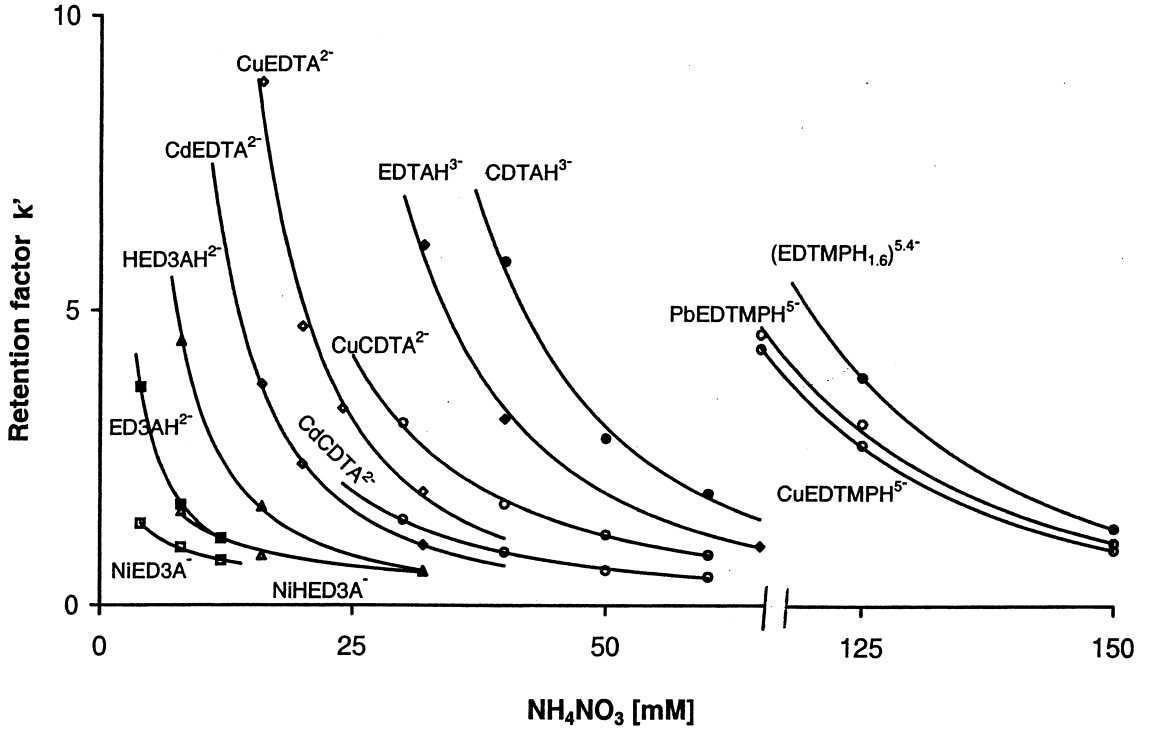

Fig. 3. Retention factors k' for EDTA and the EDTA analoges ED3A, HED3A, CDTA and EDTMP (filled symbols) with selected metal complexes (empty symbols).

pre-equilibrated citrate solutions were deposited on the column. Only Co^{2+} and Ni^{2+} gave distinct peaks. The same Co- and Ni-citrate species were observed when only free citrate was injected, thereby mobilising Co^{2+} and Ni^{2+} from the column. This can be explained by the rapid formation of Co^{2+} and Ni^{2+} complexes, but, due to the slower ligand exchange kinetics [43], the species could be observed contrary to the other metal citrate complexes, which decomposed fast enough within the time scale of the chromatography.

3.3. Separation mechanism

The role of the eluting anion and hence the separation mechanism can be assigned [51,52] by evaluating the dependence of k' on the eluent concentration ([E]). A linear correlation is found [53] for a plot of $\log k'$ vs. log[E], where the considered anion is the only active eluting ion.

For uncomplexed chelators, the data from Fig. 1 are represented in a log–log plot in Fig. 5. The linearly correlated k' in Fig. 5 suggests that NO_3^- is the only eluting anion for each chelator and hence anion-exchange is the predominant separation mechanism. The same was found for the other metal complexes, as represented by MeEDTA (Fig. 2, insert). The slopes of these lines correspond to the charge ratio (LH^{x-}/NO_3^-) of the chelator anion and NO_3^-. The charge ratios found are in close agreement with the formal values (Table 3). The largest deviation occurred for EDTA, a molecule with a greater rotational flexibility than CDTA. In Fig. 5, the steady increase of the slopes with increasing NO_3^- suggests a correlation between the different charge ratios. Such a correlation is confirmed by plotting (see Fig. 6) the slopes taken from Fig. 5 versus the mean [E] for a medium k' value (2–3). All the charge ratios correlated by the same line extrapolating through one on the *y*-axis. This is additional evidence that all of these chelators are separated consistently and almost exclusively by an anion-exchange mechanism despite

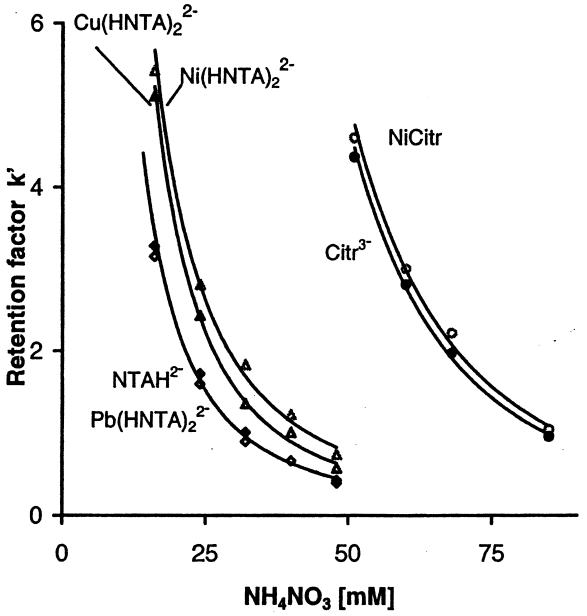

Fig. 4. Retention factors k' for the smaller chelators $(NTAH)^{2-}$ and $(Citr)^{3-}$ and metal complexes (Me = Ni^{2+}, Cu^{2+} and Pb^{2+}). Conditions as in Fig. 2.

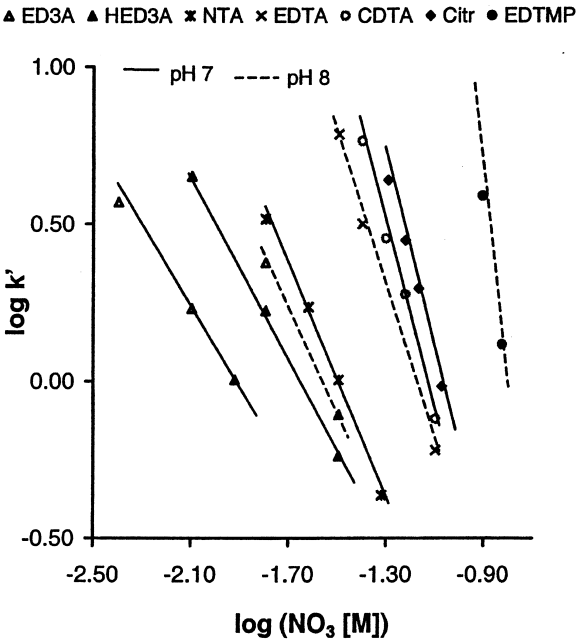

Fig. 5. Retention factors k' of free chelators (data given in Fig. 1) in a log–log plot for evaluation of the separation mechanism. Symbols same as in Fig. 1.

their different chemical compositions. Therefore, the special case of CDTA, which fits the correlation with a higher charge density than EDTA, has to be explained mainly by a higher charge density and less by hydrophobic interactions. The cyclohexane ring strongly restricts rotational flexibility compared to EDTA by blocking the negative charges on one side of the molecule.

Based on a common separation mechanism, the correlated retentions can be used to predict t_R at a given eluent concentration, or to predict the [E] needed to elute a species of known anionic charge at a desired retention time. For unknown species, the anionic charge can be estimated from the determined t_R, assuming it fits the known correlation.

3.4. Gradient separation

The chelators and their metal complexes exhibited such a high affinity for the hydrophilic anion separator that a strong eluent anion like nitrate

Table 2
Log stability constants [42] and the Me fraction (%) deposited on the column

	Co	Ni	Cu	Zn	Cd	Pb
EDTA[a]	nd	0.2%	1.8	3.1%	2.5%	0.8%
Log K_{MeEDTA}	16.3	18.6	18.8	16.5	16.5	18.0
NTA[a]	nd	2.2%	5.0%	nd	11.0%	15.0%
Log K_{MeNTA}	10.4	11.5	13.0	10.7	9.8	11.1
Citrate[a]	22.0%	45.0%	100%	nd	100%	100%
Log K_{MeCitr}	5.0	5.4	5.9	5.0	3.8	4.2

[a] An equilibrated solution of Me (0.01 mmol/L) and ligand (0.1 mmol/L) was injected, and after MeL species had appeared, EDTA was injected two to four times to fully remobilize the deposited metal. The sum of the peak integrals of mobile (MeL^{n-}) and deposited Me^{2+} (=remobil. by EDTA) equals 100%. nd, not determined.

Table 3
Measured charge ratios and formal values for some chelators and their metal complexes

Species	Measured	Formal
$NTAH^{2-}$	1.8±0.2	2
$(MeNTA)_2^{2-}$, Me=Ni^{2+}, Cu^{2+}	1.9±0.2	2
$MeEDTA^{2-}$, Me=Co^{2+}, Cu^{2+}	2.0±0.2	2
$MeEDTA^{2-}$, Me=Cd^{2+}, Pb^{2+}	1.9±0.2	2
$EDTAH^{3-}$	2.5±0.2	3
$CDTAH^{3-}$	2.9±0.2	3
$Citr^{3-}$	3.0±0.2	3
EDTMPH	5.9±0.4	5.4

provided a very high selectivity. However, as can be concluded from Fig. 1, a particular isocratic eluent concentration offers only a narrow separation window. In the same run, only species that differ by not more than one charge can be separated. In environmental waters, complexes from several chelators, including partially degraded chelators [6] (e.g. loss of a carboxylic group [54,55]), are more likely to occur. Therefore, to extend the application of the method further, gradient separation was investigated. It was found that a gradient (20–170 mmol/L NH_4NO_3, 0–8 min, pH 8) separated the

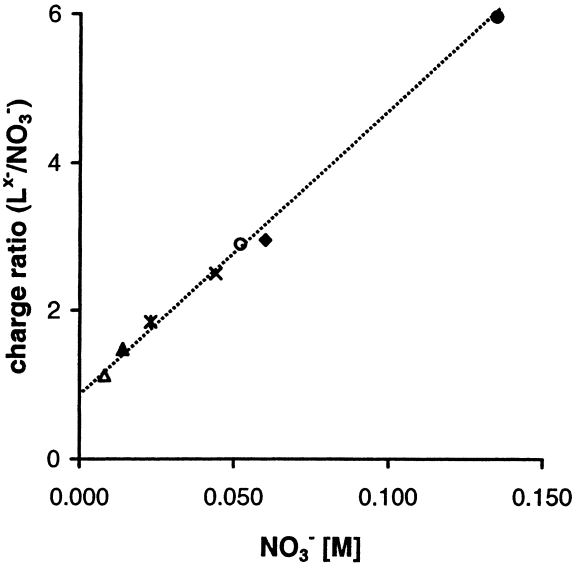

Fig. 6. Correlation of the charge ratios of free chelators and NO^{3-} (slopes in Fig. 5, listed in Table 3) plotted against the eluent concentration needed (see text) to elute the free chelator. Symbols same as in Fig. 1.

complete spectra of chelators and their complexes (depicted in Fig. 7) in the same run. The pH was crucial for the separation of free DTPA, since at pH 7.0 the dominant species $(DTPAH_2)^{3-}$ coeluted with $EDTAH^{3-}$ and at pH 7.5 it coeluted with $(CDTAH)^{3-}$. Only further deprotonation at pH 8.0 made DTPA sufficiently negative to be separated from the other two chelators. For gradient elution, a much higher separation efficiency was generally observed, yielding taller peaks. The fast measurement of isotopes by ICP-MS (~1/s) can differentiate among different metal species within incompletely separated chromatographic peaks. Therefore, there was no need to optimise the procedure to separate all the metal complexes of each chelator. Instead, the known advantages of gradient ion-exchange chromatography were used to remove the matrix and preconcentrate the sample on-column. Compared to the isocratic run, the gradient procedure results in much better detection limits and resolution. In this way, highly concentrated anionic sample components such as inorganic (Cl^-, HCO_3^-, SO_4^{2-}, 1–4 mmol/L) or buffer salts (>10 mmol/L) were eluted at the front. Using an appropriate gradient, retention times of anionic complexes were adjusted well behind this front. This is particularly important for work performed with instruments without sophisticated hardware for handling interference (high resolution or reaction cell), because elements in the concentrated matrix components can cause peaks by formation of molecular ions of the same m/z values as the heavy metals under investigation (e.g. SO^+, $ArC^+ \Rightarrow {}^{50}Cr$, ${}^{52}Cr$, ${}^{53}Cr$; ClO^+, $ClOH^+ \Rightarrow {}^{55}Mn$, ${}^{56}Fe$; S_2^+, $SO_2^+ \Rightarrow {}^{64}Zn$, ${}^{65}Cu$, ${}^{66}Zn$).

3.5. Method characteristics and applications

The gradient separation procedure was used to perform speciation analysis in diverse sample matrices and concentrations. The required stability of a Me complex ($\log K > 10$) to survive the chromatography is well below the stability constants for stable heavy metal species determined in environmental water. Hence, the applicability mainly depends on the sensitivity and the preconcentration capability of the procedure. The sensitivity depends on many factors, mainly on isotope background levels, abundance, ionisation efficiency and interference. Typical

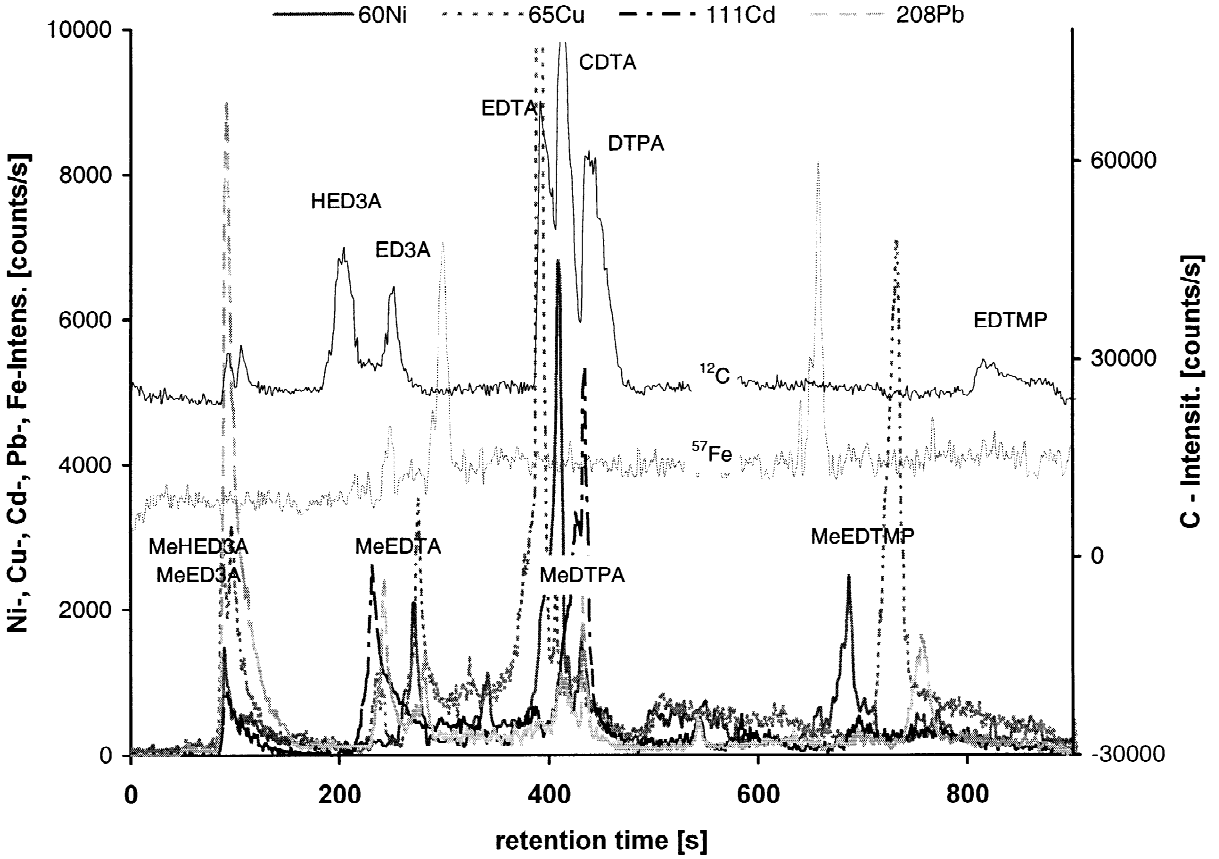

Fig. 7. Gradient separation of important aminopolycarboxylic acids (50 μmol/L each) and some of their metal complexes formed on-column. Single MeL were identified by injection of standard solutions. The high background signal at m/z 12 and 57 causing a high detection limit is explained in Section 3.5.

reproducibility and detection limits (DL) obtained for selected isotopes are listed in Table 4. The high detection limit for carbon was due to the carbon content of the Ar gas (CO_2, CH_4) and the poor ionisation efficiency in the plasma, whereas the high level of interfering molecular ions (ArN^+, ArO^+, $ArOH^+$) on the most abundant iron isotopes caused the high DL of iron. However, DLs in the lower nanomolar region and below were achieved for all other heavy metals. The lowest DLs were particularly obtained for mono isotopic elements (represented by Co in Table 4). Preconcentration of metal species on the column also strongly influences the sensitivity. This effect resulted in a smaller eluted peak volume than the volume injected and depends on the anion content of the sample and the affinity of the species for the column. The lower the anion content acting as eluting anion, the greater the preconcentration. This explains the larger volumes needed for the preconcentration of real samples (see Table 4) in order to achieve a low DL.

The method has been applied [35] in different fields of environmental research. In ecotoxicological studies of the effects of heavy metals on algae the metal speciation is critical and is therefore controlled by EDTA in the growth medium [56]. MeEDTA complexes (Me = Mn^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Zn^{2+}) in the range 0.05–1 μmol/L are analysed in nutrient broth. After filtration, loop injection allows direct observation of all the metal species for the first time without interference from high (buffer) salt concentrations.

In groundwater infiltrated by a polluted river, mobile metal EDTA species were identified and

Table 4
Reproducibility (RSD) and detection limit (DL) for EDTA and its metal complexes

Sample	Inj. vol. (mL)	Species	Isotope detected	RSD (%) (*n*) of peak areas	DL[d] (n*M*)
Standard[a]	0.27	EDTA	^{12}C	2.0 (3)	15 000
	0.27	FeEDTA	^{57}Fe	4.8 (4)	125
	0.27	CoEDTA	^{59}Co	2.5 (5)	0.8
	0.27	CuEDTA	^{65}Cu	8.0 (3)	5.0
	1.0	CuEDTA	^{65}Cu	3.4 (6)	1.0
Algae media[b]	0.27	FeEDTA	^{57}Fe	5.6 (6)	150
	0.27	CoEDTA	^{59}Co	2.8 (6)	4
	0.27	CuEDTA	^{65}Cu	3.0 (6)	5
	0.27	ZnEDTA	^{66}Zn	3.6 (6)	8
Ground water[c]	3.0	CuEDTA	^{65}Cu	6.8 (3)	2

[a] In purified water.
[b] Anions [concentrations (m*M*)]: HCO_3^- (1.2), SO_4^{2-} (0.15), NO_3^- (1.0), Cl^- (1.0).
[c] Anions [concentrations (m*M*)]: HCO_3^- (4.5), SO_4^{2-} (0.8), NO_3^- (0.3), Cl^- (1.1).
[d] Given as 3σ [57].

determined by standard addition [35]. In river water samples (5 mL preconc.) from sites polluted to different levels, other anionic Cu complexes were found to coexist with CuEDTA at concentrations of 1–2 nmol/L.

4. Conclusions

In a search for the optimal conditions for the coupling of anion-exchange chromatography and ICP-MS, the properties of ammonium nitrate were found to provide an optimal integration of both techniques. The thermal instability of the eluent salt makes it the best choice for the plasma and its affinity for low hydrophobic anion exchangers provides either high selectivity (isocratic) or a broad analytical window (gradient) for anionic species. Several of the most important aminopolycarboxylic chelators and their metal complexes were separated by an anion-exchange mechanism with only a minor contribution from hydrophobic interactions. This also enables the method to predict the charge of analogous species.

The separation is susceptible to small structural features, such as the charge at a given pH and the size in combination with the composition of the chelator molecule and its metal complex. In contrast to the other ion chromatographic procedures used, the eluent pH is decoupled from the eluent strength and can be adjusted in a broad range, including around neutral. This provides very important advantages: species can be chromatographed at their original sample pH, and the eluent strength can be adjusted independently to the eluent pH. Alternatively, the eluent pH can be used to control the species charge by the degree of protonation and hence additionally assist in selectivity control.

All these features contribute to the potential of the described anion-exchange separation combined with ICP-MS, for sensitive isotope-specific element detection, of becoming the method of choice for fast and selective speciation analysis of anionic metal species of a certain stability.

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