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High performance anion chromatography of gadolinium chelates

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Abstract

High performance anion chromatography (HPIC) method to separate ionic Gd chelates, $[GdDTPA]^{2-}$, $[GdEDTA]^{-}$, $[GdDCTA]^{-}$ and free matrix anions was developed. At alkaline pHs, polydentate complexing agents such as ethylene-diamine-tetraacetate (EDTA), the diethylene-triamine pentaacetate (DTPA) and trans-1,2-diamine-cyclohexane-tetraacetate (DCTA) tend to form stable Gd chelate anions and can be separated by anion exchange. Separations were studied in the simple isocratic chromatographic run over the wide range of pH and concentration of carbonate eluent using suppressed conductivity detection. The ion exchange and complex forming equilibria were quantitatively described and demonstrated in order to understand major factors in the control of selectivity of Gd chelates. Parameters of optimized resolution between concurrent ions were presented on a 3D resolution surface. The applicability of the developed method is represented by the simultaneous analysis of Gd chelates and organic / inorganic anions. ICP-AES (inductively coupled plasma atomic emission spectroscopy) analysis was used for confirmation of HPIC results for Gd. Collection protocols for the heart-cutting procedure of chromatograms were applied. SPE procedures were also developed not only to extract traces of free gadolinium ions from samples, but also to remove the high level of interfering anions of the complex matrices. The limit of detection, the recoverability and the linearity of the method were also presented.

Keywords: anion chromatography, complex stability, gadolinium chelates, ion exchange equilibria, optimized separation

Introduction

Lanthanide complexes are of great interest due to their coordination behavior in analytical and environmental chemistry and their use as contrast agents in magnetic resonance imaging. Paramagnetic contrast agents as gadolinium chelates can be of assistance to further increase the discrimination between clinically important information [1]. However, Gd has the potential of leeching into membranes and enzymatic structures, causing as-yet undetermined long-term consequences. On the other hand, Gd is among the most important emerging environmental contaminants in hospital effluents and surface waters [2]. The ionic radius of Gd(III) is very nearly equal to that of divalent Ca. This is one of the reasons why Gd is so toxic in environmental systems. As discussed in the literature [3], the toxicity of these complexes, and consequently their applicability is strictly connected with their thermodynamic stability in aqueous solution. Transmetallation of Gd complexes leads to release of free gadolinium through replacement of the Gd(III) within the chelate molecule by pollutant cations such as zinc, copper or iron [4].

Metal complexed anions vs. free inorganic or organic anions are often useful information in environmental and bioanalytical studies. In recent years, there has been increasing interest in analysis of Gd compounds. A basic method of determination was developed for gadolinium using inductively coupled plasma atomic emission spectroscopy (ICP-AES) [5]. Several papers on the ICP spectroscopy method have been published, most of these involve the use of hyphenated techniques (CE/ESI-MS, HPLC/ICP-MS, HILIC/ICP-MS) [6, 7, 8]. A valuable concept about the potentially metabolic pathways of most frequently used Gd chelates was presented by Telgmann et al. [9] using electrochemistry/capillary electrophoresis/ESI-MS or ICP-MS in tandem system. It is a possible analytical platform with the labor-intensive procedure, but the complexity for routine analysis is currently high and expensive.

However, speciation analysis of Gd complexes requires not only an element selectivity method as the ICP-AES but differentiation of Gd species (chelate complexes, matrix anions, co-ions) is fundamental. Selective and effective chromatography must be used for Gd based compounds that contains different chemical forms of Gd species which show changes in charge and different biological and environmental activity by pH of solutions. Moreover, the pH value must be rather accurately known, since a small shift in pH can considerably alter the value of the conditional stability of metal and protonation of ligands. The utility of liquid chromatography of Gd compounds was demonstrated with RP-HPLC and HILIC methods using UV detection. The resultant systems have ability to separate complexes, whilst different matrix ions and ligands cannot be separated. In addition a disadvantage of HILIC with ICP-MS is the high input of organic solvents, which may cause the formation of carbon deposits due to the combustion.

One of the most effective developments in ion chromatography in the past decade is the introduction of a procedure that allows the simultaneous analysis of metal complex anions and organic / inorganic anions. Mechanism and models for simultaneous separation of transition metal chelate complexes and ligands have been developed by us [10, 11, 12] using latex based stationary phase and suppressed conductivity detection in high performance anion exchange chromatography.

The aim of this paper was to develop an effective and selective ion chromatographic method of Gd chelates and matrix anions. The paper will describe the mechanism of retention based on complex formation- and ion exchange equilibria. This article will also be concentrate on the criteria for eluent composition and retention controlling to provide an optimized resolution between the Gd chelates and inorganic or aliphatic organic anions.

Theory

Complex formation and protonation equilibria of gadolinium chelates in anion chromatography

At alkaline pHs, polydentate complexing anions, such as polyaminocarboxylic acids (EDTA, DCTA, DTPA, etc.), tend to form stable chelate complexes with most of the di- and trivalent metal cations. When basic solution contains an excess of strong complexing anion of high charge (L^{n-}), metal ions will occur as anionic complexes and can be separated by anion exchange [Eq. (1)]. Hence this method provides simultaneous metal and anion separation.

$$L^{n-} + Gd^{3+} \rightleftharpoons [GdL]^{z-} \quad (1)$$

where $z = 3 - n$.

However, trivalent lantanide cations including Gd differ from the more common transition metals in having large coordination numbers, usually as hydrated ion with inner sphere water molecules. The ethylene-diamine-tetraacetate (EDTA), the diethylene-triamine pentaacetate (DTPA) and trans-1,2-diamine-cyclohexane-tetraacetate (DCTA) are strong chelating agents able to form sufficiently stable complexes with Gd(III) ions.

The resulting Gd complexes may have a net charge, depending on the nature of ligands involved in the complex formation and the conditions used. An important factor in the selection of eluent pH is its compatibility with the complex formation. In Fig. 1, the protonation and complex formation equilibria of Gd-DTPA can be seen calculated by corresponding conditional equilibrium constans (Table I) using Medusa-Hydra chemical equilibrium software (KTH Royal Institute of Technology, 2004). As it can be seen in Fig. 1, different species of Gd(III) can exist in a solution depending on the pH. The figure shows clearly that at the typical alkaline pH range used during anion separations (pH 9 - 12) the

dominant species is $[GdDTPA]^{2-}$ in the solution. All the Gd(III) is in complexed anionic form both at the pH of elution and the pH of suppressed conductivity detection. It makes the separation of the chelate anion possible by the means of anion chromatography. This is due to the high complexing power of the ligand with the gadolinium ion. Molar distribution provides very similar diagrams of looking the equilibria and consequences for $[GdEDTA]^-$ and $[GdDCTA]^-$ complexes. Fig. 1 also shows, that $Gd(OH)_3$ precipitate forms above pH 12, suggesting that the use of carbonate/bicarbonate eluents are preferred over NaOH for separation of Gd(III) chelates. Maintaining of conditions under the analytical procedure is of particular importance. In this case the complex stability and protonation are not accompanied by a significant structural change of the original sample.

Ion exchange equilibria of gadolinium chelates in anion chromatography

If the gadolinium chelate has a net negative charge it can be separated by the means of anion chromatography. The basis of separation is the ion exchange between the eluent and the chelate anions. The selection of appropriate composition of eluent is crucial to the success of IC separation. The problem in selection is magnified when simultaneous separations of metal complex anions and simple anions are to be performed. In ion chromatography, two types of mobile phases are used mainly: hydroxide (NaOH, KOH) and carbonate/bicarbonate (Na_2CO_3) based eluents. The advantage of carbonate eluent is the manipulation of the selectivity by two components (bicarbonate and carbonate) which provides practical combination of eluting power. Additional advantage is the reproducible eluent concentrations, compared to the more laborious of carbonate-free hydroxide eluents. The latter one contains at least three distinct competing eluent anions, the divalent carbonate (CO_3^{2-}), and the

monovalent hydrogen carbonate (HCO_3^-) and hydroxide (OH^-) ions. For the description of elution behavior of metal chelate complexes when carbonate eluent is used as eluent, the following independent equilibria should be taken into account:

$$z\,R-HCO_3^- + [GdL]^{z-} \overset{K_{GdL/HCO_3^-}}{\rightleftharpoons} R_z-GdL + z\,HCO_3^- \quad (2)$$

$$z\,R-OH^- + [GdL]^{z-} \overset{K_{GdL/OH^-}}{\rightleftharpoons} R_z-GdL + z\,OH^- \quad (3)$$

$$z\,R-CO_3^{2-} + [GdL]^{z-} \overset{K_{GdL/CO_3^{2-}}}{\rightleftharpoons} R_z-GdL + z\,CO_3^{2-} \quad (4)$$

where *R* denotes the anion exchange stationary phase that has been conditioned with eluent components and *K* is the equilibrium constant of appropriate ion exchange reaction. The following ion exchange equilibria are also considered between the competing eluent anions [13]:

$$2\,R-HCO_3^- + CO_3^{2-} \overset{K_{CO_3^{2-}/HCO_3^-}}{\rightleftharpoons} R_2-CO_3 + 2\,HCO_3^- \quad (5)$$

$$R-HCO_3^- + OH^- \overset{K_{OH^-/HCO_3^-}}{\rightleftharpoons} R-OH + HCO_3^- \quad (6)$$

Accordingly, several factors affect the separation of chelate anions on anion exchanger. These are the simultaneous complex formation, protolysis and ion-exchange equilibria [Eqs. (2) - (6)]. Here, the concentration and form of a given species is chemically altered to affect the retention and separation selectivity. In this case retention equations can be derived

which predict elution behavior using the multispecies retention model. A detailed mathematical and chemical description can be found in our earlier paper [10, 11, 13]. Based on these relations, it is possible to choose the optimal elution condition for Gd chelates. In the Experimental part we have presented practical considerations in this matter.

Experimental

Materials

For preparing eluents and standard solutions high purity ($18.2\ M\Omega\ cm^{-1}$) deionized water purified by Milli-Q system (Millipore, Bedford, MA, USA) containing a 0.22 µm Millistack filter at the outlet was used. The 20 mM concentration stock solutions of ethylene-diamine-tetraacetate (EDTA), and trans-1,2 diamino-cyclohexane-tetraacetic acid monohydrate (DCTA), the diethylene-triamine-pentaacetate sodium salt (DTPA), $GdCl_3$, lactate, succinate, maleate were prepared by dissolution of analytical grade salts $Na_2EDTA \times 2H_2O$, $CH_3CH(OH)COOLi$, $NaOOC(CH_2)_2COONa \times 6H_2O$, $NaOOCCH=CHCOONa$, $GdCl_3 \times 6H_2O$ from Sigma-Aldrich and NaCl, NaBr, NaNO $NaNO_3$, Na_2SO_4, Na_3PO_4 and H_2SO_4 from Fluka Chemie AG, Buchs. The standard mixtures were prepared by the mixing and dissolving of stock solutions and stored in polypropylene bottles. The mobile phase composition was varied depending on the experiments (9.8 < pH < 10.8 and 3.0mM < carbonate concentration < 4.5mM) and details are given in the text and figures. For extraction and matrix elimination studies the Phenomenex Strata-SCX SPE ion exchange cartridge (200 mg sulphonated resin, 1 mequiv/g capacity) was used. For quantitative determinations of

preconcentrated Gd the cartridge was eluted by 0.2 mM $Na_5(DTPA)$ at pH = 11. The eluent was degassed in ultrasonic bath (Sonorex RK 52, Badelin) for 10 min before use.

Instrumentation

All measurements were performed with a Dionex DX300 gradient chromatographic system (Dionex, Sunnyvale, CA, USA) that consists of a CHA-6 high pressure chromatographic module, Dionex EDM eluent degas module and gradient pump equipped with a conductivity detector CDM-II. Chromatograms were recorded digitally using Dionex ACI advanced computer interface and Dionex AI 450 software. Model 9125 injection unit (Rheodyne, Rohnert Park, CA, USA) was applied containing 50 µl injection loop.

Separations were carried out by a Dionex IonPac AS4A-SC polymer-based anion-exchange separator with alkanol amine functional groups. It is a low-capacity carbonate eluent anion-exchange column for the fast, isocratic separation. The ion-exchange capacity of the column was 20 µequiv./column. The separator column (250×4 mm) was based on a 13 µm polystyrene-divinylbenzene co-polymer agglomerated with completely aminated anion exchange latex. The column was equilibrated with the degassed mobile phase.

Chemically suppressed conductivity detection was accomplished using a Dionex AMMS-II micromembrane suppressor which was continuously regenerated with 0.025 M sulfuric acid with a flow rate of 3.5 ml/min. All samples were analyzed in triplicate with a flow rate of 1.2 ml/min and regression analysis was carried out using mean values of conductivity intensities. Data processing and calculations were performed by using Peakfit version 4.12, Wolfram Mathematica 10.0, and MS Office Professional Edition 2010 softwares on a computer equipped with Intel Core i7 CPU running GNU/Linux operating system (Debian Jessie).

For ICP-AES analysis a Spectroflame Modula E optical plasma interface (OPI) instrument was used. The instrument was equipped with an axial end-on-plasma torch (SPECTRO Analytical Instruments Inc., Germany). The detection wavelength was 303.284 nm.

Results

Control of ~~peak positions~~ retention of Gd chelates

The position of individual peaks of complex sample of chelates can be identified by the injection of different Gd complexes, respectively. The Fig. 2 (a, b, c) shows the ~~retention position~~ chromatograms of Gd chelates with three key complexing ligands that have been studied.

The retention of ionic compounds increases according to the empirical sequence of the ligands: $[\mathrm{GdDTPA}]^{2-} < [\mathrm{GdEDTA}]^{-} < [\mathrm{GdDCTA}]^{-}$. The applicability of described method is demonstrated in Fig. 2 (d), which shows selective separations of three different Gd chelates and chloride. Separations were studied in the simple isocratic run over the wide range of pH and concentration of eluent. Fig. 2 (d) also shows the increased affinity of Gd(DCTA) relative to another two Gd complexes. The DCTA ligand forms more lipophilic complexes than does EDTA or DTPA and so has greater retention. The multispecies eluent - analyte retention model [10] has been applied to predict retention data of $[\mathrm{GdEDTA}]^{-}$ and $[\mathrm{GdDTPA}]^{2-}$. The unknown selectivity parameters (*K*, ion-exchange selectivity coefficients) of model equations for chelates were determined from the wide range of experimental retention data by iterative minimization, using a non-linear regression algorithm ($K_{GdDTPA/OH} = 6.04$ and $K_{GdEDTA/OH} = 25.36$). The results in 3D retention surfaces are presented in Fig. 3 and

demonstrate that the retention data are influenced strongly by pH for $[GdEDTA]^-$. There are however relatively large retention gaps between two complexes. Accordingly, ion chromatography has a great analytical potential for selective separations of chelate components.

It is important to note that, although, the charge is one of the main factors that govern the ion exchange retention, other factors have significant effect, as well. The retention order of complex ions is also determined largely by their radius, geometrical structure, solvation and additional exclusion effects. Attention must be paid to the fact that the Gd chelate complexes are fundamentally different chemical entities. EDTA forms octahedral chelates with metals while DCTA ligand forms even more stable complexes with metals by cyclohexyl ring. As a result, the affinity of these components toward the ion exchanger differs significantly.

Identification of gadolinium in the peaks

Atomic plasma spectroscopy (ICP-AES) using heart cutting portion of effluent from ion chromatograph can provide a confirmation of Gd presents in peaks of complex samples. Heart cutting techniques, wherein a designated portion of the IC column effluent containing target ions ($[GdDTPA]^{2-}$ and $[GdEDTA]^-$) is collected and used to further ICP analysis, represent a powerful approach. Because of the pH dependency of metal coordination and complex stability the effluent was collected directly after the separation column and before the suppressor device (pH > 7). Collection protocols for this procedure were applied in the analysis of Gd containing peaks. The sufficient time window of the collection of effluent was applied in the retention interval of 2.8 - 5.5 min for $[GdDTPA]^{2-}$ and 18.5 – 23.5 min for $[GdEDTA]^-$, respectively (Fig. 4). The collected fractions were analyzed by ICP-AES

technique. From Fig. 4 it is quite obvious that the second and third peaks come from Gd complexes. The data presented in Fig. 4 confirms the selective separation of Gd(III) chelates and the proper identification of chromatographic peaks. In fact, the Gd containing species separated by ion chromatography can be accurately confirmed via ICP in the peaks, on another experimental basis, because it did not respond to organic ligands or eluents.

Discussion

Method development for matrix effects

The success of ion chromatography is due primarily to the ongoing evolution of high selectivity of ionic separations in complex matrices. However, overcoming the sparse matrix effects is necessary to achieve optimized separations. In the case of species considered in this study, the matrix effects of anions must also be taken into consideration. Some authors described that destabilization of the $[GdEDTA]^-$ and $[GdDTPA]^{2-}$ can occur through phosphate anions [14]. Interestingly, the observed values of ion exchange selectivity data of HPO_4^{2-} and $[GdDTPA]^{2-}$ are in very similar interval in this separation system ($K_{HPO_4/OH} = 5.55$ and $K_{Gd-DTPA/OH} = 6.04$). The multiple species retention modeling (see in [10]) gives sufficiently retention data to serve as the basis of an optimization procedure of eluent composition and elimination of matrix effect. We used a relatively large number of data points for HPO_4^{2-} and $[GdDTPA]^{2-}$ ions and we can predict retention behavior with optimized resolution between two concurrent ions at the wide range of eluent conditions (9.8 < pH < 10.8 and 3.0mM < carbonate concentration < 4.5mM, Fig. 5). The change in the molar ratio of eluent / analyte components as a function of pH results a significant change in the resolution. The ion exchange phenomenon is in accordance with Fig. 5 which shows a

resolution surface, i.e. the R_s vs. pH functions at different eluent concentrations have their maxima in the range pH = 11 − 12. The grey area represents the standard values of resolution, R_s = 1.3. This is the consequence of the difference between the retention behavior of two analyte anions and also between the protonation of carbonate and phosphate anions. Figures 6 (a, b) show optimized chromatograms for the typical separation of different organic / inorganic anions and $[\mathrm{GdDTPA}]^{2-}$ complex anions.

Solid phase extraction technique (SPE) became a vital pre-concentration and separation method. SPE procedures can be used used not only to extract traces of gadolinium ions from samples, but also to remove the high level of interfering anionic components of the complex matrices. A known amount of $GdCl_3$, NaCl, NaBr, $NaNO_3$ and Na_2SO_4 was injected onto the cationic SPE cartridge under acidic condition. At this pH range gadolinium will practically completely enter the sulfonated cationic resin phase and anions remain completely in the aqueous phase and remove from the complex matrix. For total elimination of interfering anions the cartridge was washed successively with high purity water. A benzene sulfonic acid group (R-SO3-) is bonded to the surface of the SCX-SPE silica particle, giving strong cation-exchange selectivity for Gd(III). See also in Eq. (7) and Fig.7(b). Evidence pointing to interaction between the fixed sulfonic acid groups and counter cations is the cationic equilibrium. Effluents were controlled for appropriate washing by anion chromatography (see Fig. 7 (a)). For quantitative determinations of preconcentrated Gd the cartridge was eluted by 0.2 mM $Na_5(\mathrm{DTPA})$ at pH = 11, based on simultaneous ion exchange and complex formation equilibria (Eq. 7).

$$(R-SO_3)_3\,Gd + DTPA^{5-} + 3\,Na^+ \overset{K_{spe}}{\rightleftharpoons} 3\,R-SO_3\,Na + [GdDTPA]^{2-} \quad (pH \approx 11) \quad (7)$$

A sufficient elution is evident, since $K_{spe} = K_{iex,Gd/Na} \times K'_{stab,GdDTPA} \approx 10^{22}$, where $K_{iex,Gd/Na}$ is the ion exchange equilibrium constant and $K'_{stab,GdDTPA}$ is the conditional stability constant at pH ≈ 11. The recoverability of Gd was determined using proposed chromatographic method. The $[GdDTPA]^{2-}$ was collected before and after loading onto the cartridge. The peak appears with retention time of 3.4 min (Fig. 7 (b)). The peak of $[GdDTPA]^{2-}$ is symmetric and clearly distinguishable from the baseline. After triplicate injections, percent recovery value was calculated and was given as 91 %.

Limit of detection and linearity of the method

The release of Gd into the human body is of significant interest also in clinical chemistry. Due to the varying toxicity of the particular Gd^{3+} species it is important to not only investigate total Gd^{3+} concentrations, but the Gd species as well. One of the most active Gd^{3+} based MRI agents is the $[GdDTPA]^{2-}$. For these reasons there is continuing interest in the data analysis of this component. To show the analytical applicability of developed method, the chromatographic determination of a sample of $[GdDTPA]^{2-}$ was validated. The LoD is determined from the slope of calibration (*m*) and the root mean square error (*RMSE*) calculated from the differences of the measured calibration points and the calibration curve. The low concentration range of $[GdDTPA]^{2-}$ was varied from 0.5 to 5.0 µM and the peak area of conductivity vs. concentration data was calculated. The detection limit defined as $3 \times RMSE/m$ [17] has been estimated as 0.33 µM which corresponds to 15.4 ng $[GdDTPA]^{2-}$ in a 50 µL injection loop. The calibration plot was linear and the correlation coefficient (r^2) was 0.9887. Analytical data for monitoring Gd complexes by ion

chromatography in natural water are still lacking, however IC includes a variety of detection modes [16]. The curve has a good linearity and a relatively large sensitivity. Accordingly, the Gd chelate anions can be determined without using any preconcentration techniques at trace levels by the means of anion chromatography using suppressed conductivity detection and low-capacity carbonate selective anion-exchange column.

Evaluation of the method for spiked water sample

Figure 8 shows the chromatograms of direct injection of drinking water spiked with $[GdDTPA]^{2-}$ at 0.18 mg/L. Area of practical separation of Gd chelate is highlighted by retention interval of 4.5 – 6.0 min. The target analyte Gd chelate anion was well resolved from the sample matrix. The benefits of method for $[GdDTPA]^{2-}$ separation can clearly be seen in Figure 8, where the chelate peak response is significantly enhanced. Quantitative recovery (96.8 %) was obtained for the added chelate anion in drinking water matrix.

Conclusions

A high performance selective alternative for the analysis of Gd(III) is to use ion chromatography combined with complex equilibria as presented in this work. The developed efficient analytical method generates high quality data by the selective and simultaneous separation of Gd(III) chelates, organic and inorganic anions in a short analysis time. Chromatographic behavior of Gd chelate anions and retention data to support the theory and practice of separation are presented here to demonstrate the applicability of the proposed method. This procedure is a simple, selective and isocratic chromatography for the simultaneous analysis of Gd chelate complexes and free anions in organic/inorganic matrices.

Separation equilibria were quantitatively described and the control of selectivity is demonstrated. The study clearly shows appropriate solutions to problems of peak identification and optimization of separation. These trends point to utility of HPIC method capable a total anion separation for the similar sample composition. Collection protocols of chromatograms of Gd chelates for ICP spectroscopy and solid phase extraction method were also developed in this study. It was demonstrated that by applying solid phase extraction, all the anionic matrix ions can be removed and the chelates of Gd(III) can be analyzed without any anionic interference. To the best of our knowledge, this article is the first instance for description and fundamental study of simultaneous separation of Gd-chelates using high performance anion chromatography in the literature.

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Tables

Table I: Stability constants of Gd chelates.

GdL	log KL[a]	pH 7.4		pH 10.0	
		$\alpha_{L(H)}$	log K'L[b]	$\alpha_{L(H)}$	log K'L[b]
$[GdEDTA]^-$	17.4	2.8	14.6	0.5	16.9
$[GdDCTA]^-$	19.6	4.5	15.1	1.8	17.8
$[GdDTPA]^{2-}$	22.5	4.4	18.1	0.7	21.8

[a] Overall thermodynamic stability constants [15]

[b] Calculated conditional stability constants at physiological- and eluent pHs

Figure captions

Figure 1. Molar fractions of GdL species as a function of pH. The grey area represents the appropriate pH of elution and detection.

Figure 2. Separations of anionic Gd chelates (a, b, c, d). Column: AS4A-SC, Eluent: 7.2 mM carbonate buffer. Sample: (1) Chloride 1 mM, (2) Gd-DTPA 1 mM, (3) Gd-EDTA 1 mM, (4) Gd-DCTA 1 mM.

Figure 3. Calculated retention surfaces for $\left[\mathrm{GdEDTA}\right]^{-}$ and $\left[\mathrm{GdDTPA}\right]^{2-}$ complexes eluted with carbonate buffer $\left(\mathrm{C} = \left[\mathrm{HCO_3^-}\right] + \left[\mathrm{CO_3^{2-}}\right]\right)$.

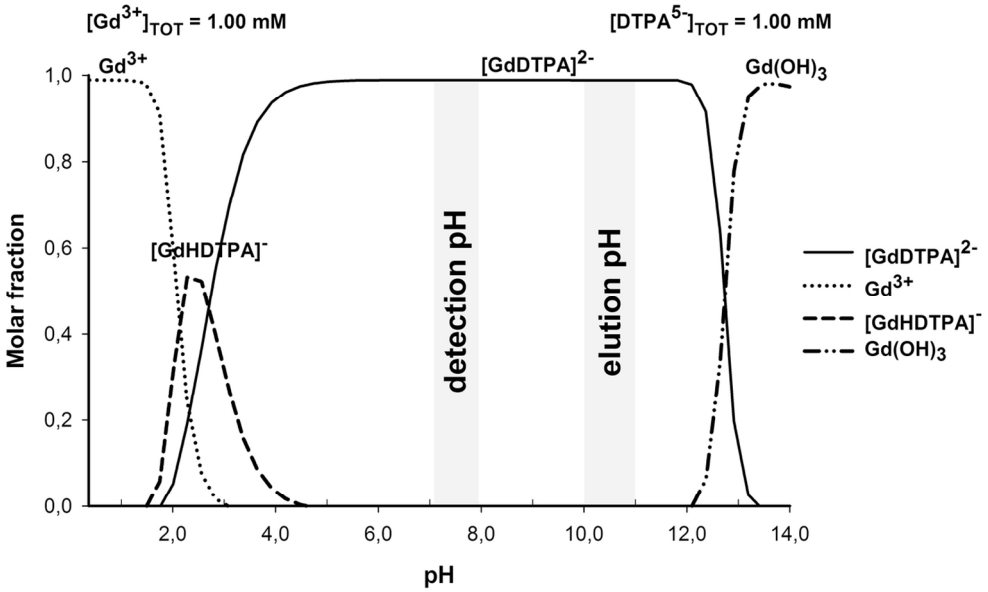
Figure 4. The identification of Gd containing peaks by ICP-AES in ion chromatographic separation of Gd chelate complexes using heart cutting portion of effluent from ion chromatogram. Eluent: 3.5 mM carbonate buffer, pH = 10.2. Sample: Gd-DTPA 1.0 mM, Gd-EDTA 1.0 mM.

Figure 5. Calculated resolution surface between phosphate and $\left[\mathrm{GdDTPA}\right]^{2-}$ ions eluted with carbonate buffer $\left(\mathrm{C} = \left[\mathrm{HCO_3^-}\right] + \left[\mathrm{CO_3^{2-}}\right]\right)$. Partial molar fractions of carbonate components together with the $\mathrm{OH^-}$ concentration and those of phosphates are also illustrated as functions of pH. The grey area represents the standard values of resolution, $R_s = 1.3$.

Figure 6. (a) Separation of organic anions and $\left[\mathrm{GdDTPA}\right]^{2-}$. Eluent: 3.5 mM carbonate buffer, pH = 10.2. Sample: (1) Lactate 0.5 mM, (2) Chloride 0.1 mM, (3) Gd-DTPA 0.01 mM, (4) Succinate 0.03 mM, (5) Maleate 0.15 mM. (b) Separation of inorganic anions and $\left[\mathrm{GdDTPA}\right]^{2-}$. Eluent: 3.0 mM carbonate buffer, pH = 10.5. Sample: (1) Chloride 0.1 mM, (2) Nitrate 0.02 mM, (3) Gd-DTPA 0.01 mM, (4) Sulphate 0.01 mM, (5) Phosphate 0.05 mM.

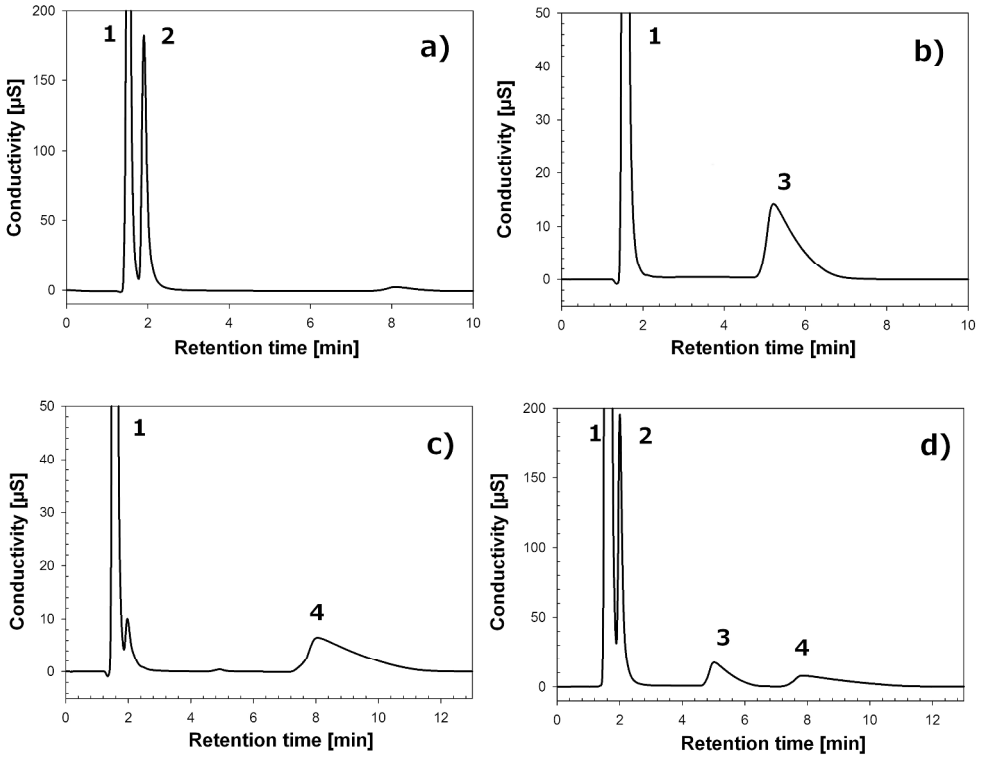
Figure 7. (a) Chromatograms of inorganic matrix anions under the cleaning of SPE-SCX cartridge with washing by high purity water (mL). (b) Elution and recoverability of 0.05 mM $[GdDTPA]^{2-}$ from the SPE cartridge (see also in Eq. 7).

Figure 8. Separation of Gd chelate anions $[GdDTPA]^{2-}$ in spiked drinking water (0.18 mg/L). Eluent: 2 mM Na_2CO_3 / $NaHCO_3$. Sample pretreatment: 0.45 μm filtration. Sample: (1) Chloride, (2) Nitrate, (3) Gd-DTPA, (4) Sulphate.



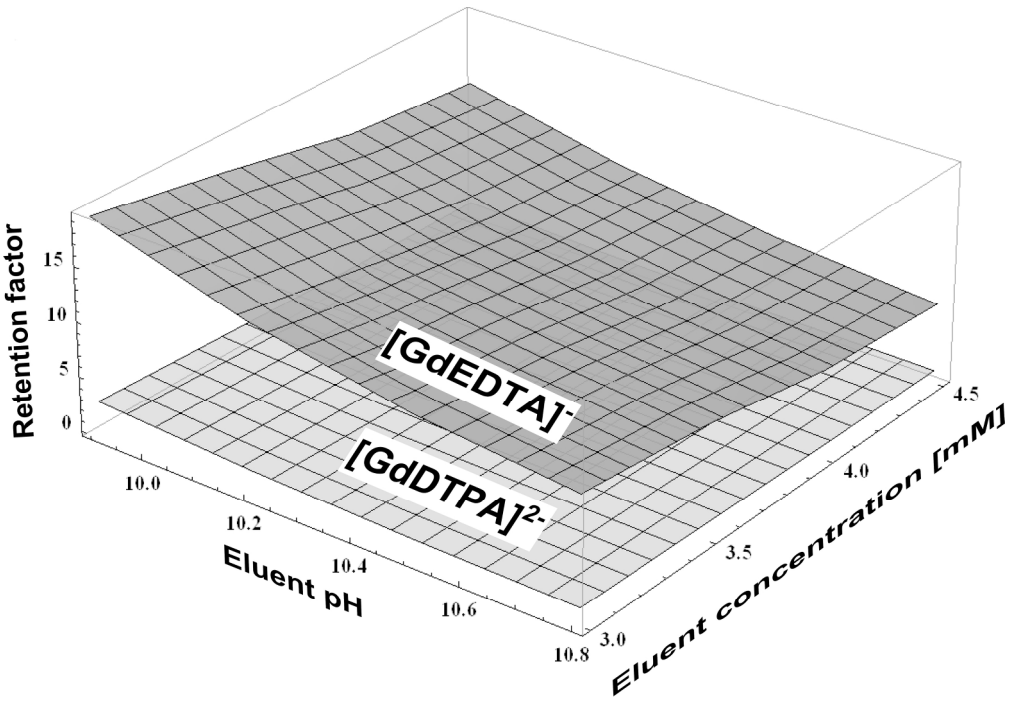
Molar fractions of GdL species as a function of pH. The grey area represents the appropriate pH of elution and detection.

124x73mm (300 x 300 DPI)



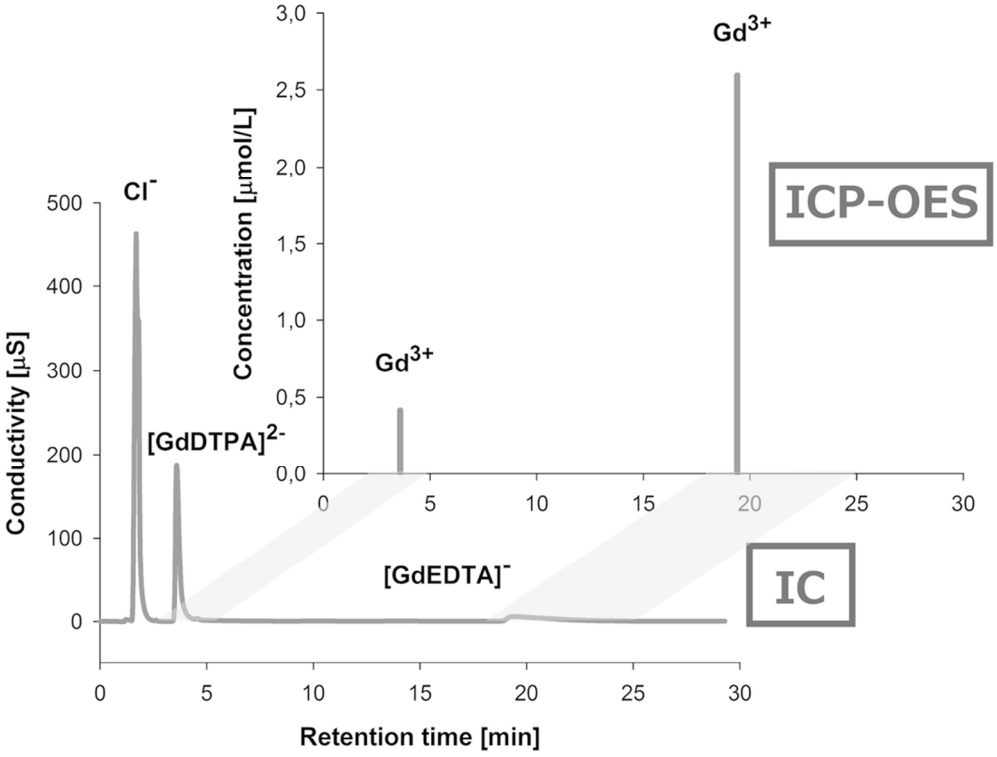
Separations of anionic Gd chelates (a, b, c, d). Column: AS4A-SC, Eluent: 7.2 mM carbonate buffer. Sample: (1) Chloride 1 mM, (2) $[GdDTPA]^{2-}$ 1 mM, (3) $[GdEDTA]^{-}$ 1 mM, (4) $[GdDCTA]^{-}$ 1 mM.

307x242mm (300 x 300 DPI)



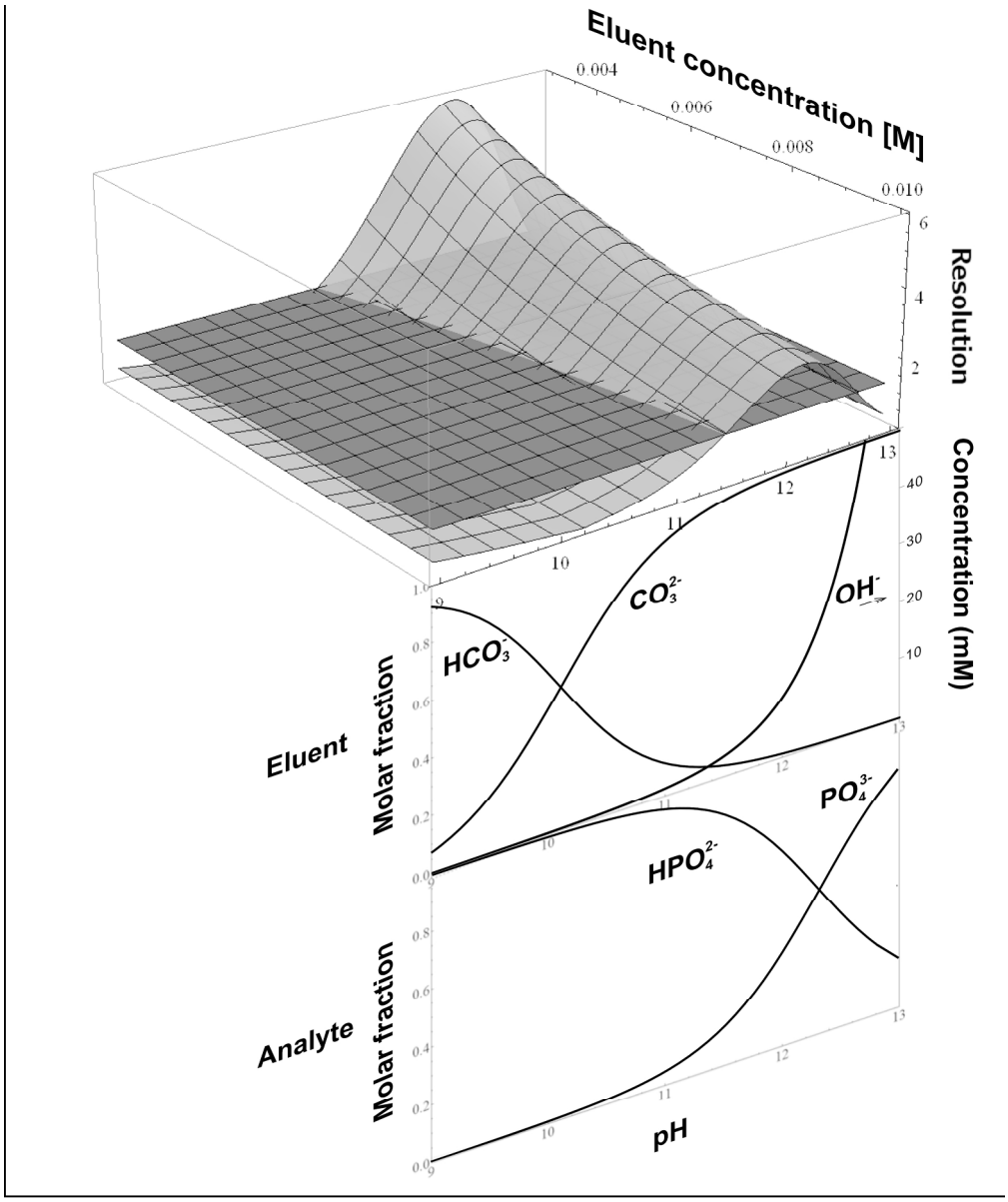
Calculated retention surfaces for $[GdEDTA]^-$ and $[GdDTPA]^{2-}$ complexes eluted with carbonate buffer (c = $[HCO_3^-]$ + $[CO_3^{2-}]$.

207x142mm (300 x 300 DPI)



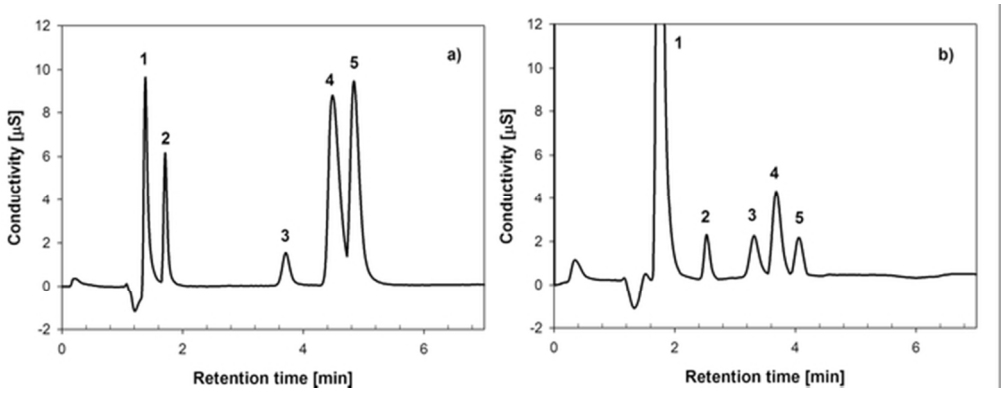
The identification of Gd containing peaks by ICP-AES in ion chromatographic separation of Gd chelate complexes using heart cutting portion of effluent from ion chromatogram. Eluent: 3.5 mM carbonate buffer, pH = 10.2. Sample: $[GdDTPA]^{2-}$ 1.0 mM, $[GdEDTA]^{-}$ 1.0 mM.

97x74mm (300 x 300 DPI)



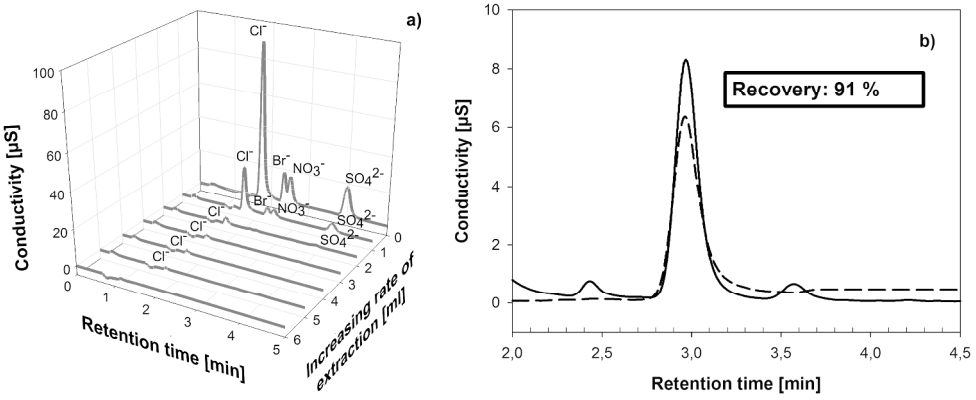
Calculated resolution surface between phosphate and $[GdDTPA]^{2-}$ ions eluted with carbonate buffer (c = $[HCO_3^-]$ + $[CO_3^{2-}]$. Partial molar fractions of carbonate components together with the OH^- concentration and those of phosphates are also illustrated as functions of pH. The grey area represents the standard values of resolution, Rs = 1.3.

211x252mm (300 x 300 DPI)



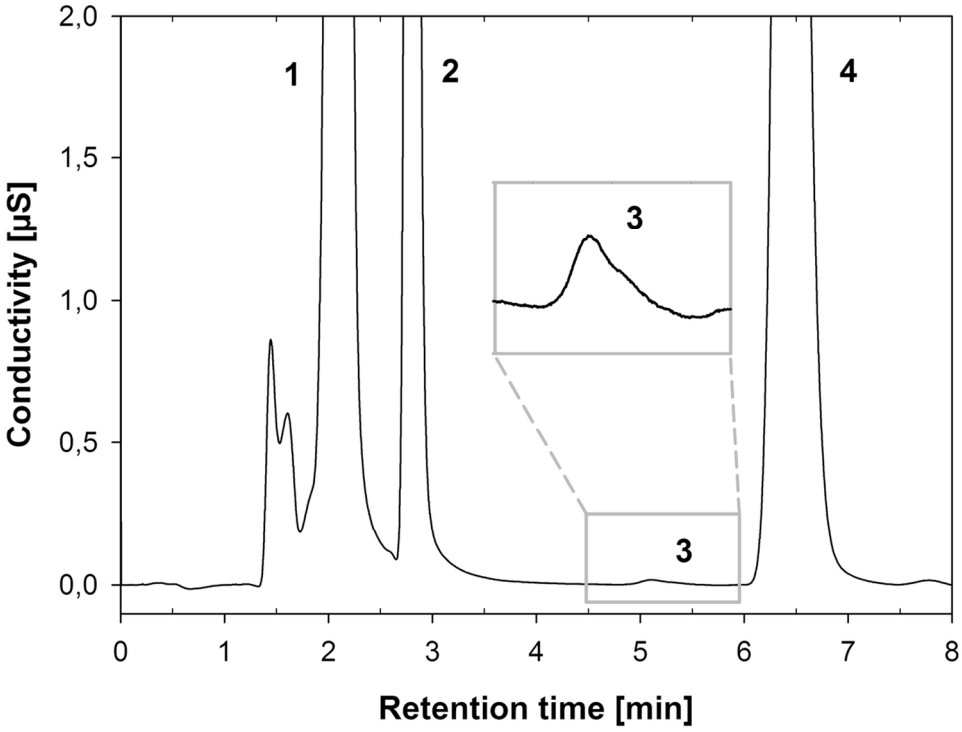
(a) Separation of organic anions and $[GdDTPA]^{2-}$. Eluent: 3.5 mM carbonate buffer, pH = 10.2. Sample: (1) Lactate 0.5 mM, (2) Chloride 0.1 mM, (3) $[GdDTPA]^{2-}$ 0.01 mM, (4) Succinate 0.03 mM, (5) Maleate 0.15 mM. (b) Separation of inorganic anions and $[GdDTPA]^{2-}$. Eluent: 3.0 mM carbonate buffer, pH = 10.5. Sample: (1) Chloride 0.1 mM, (2) Nitrate 0.02 mM, (3) $[GdDTPA]^{2-}$ 0.01 mM, (4) Sulphate 0.01 mM, (5) Phosphate 0.05 mM.

56x21mm (300 x 300 DPI)



(a) Chromatograms of inorganic matrix anions under the cleaning of SPE-SCX cartridge with washing by high purity water (mL). (b) Elution and recoverability of 0.05 mM $[GdDTPA]^{2-}$ from the SPE cartridge (see also in Eq. 7).

301x120mm (300 x 300 DPI)



Separation of $[GdDTPA]^{2-}$ anions in spiked drinking water (0.18 mg/L). Eluent: 2 mM $Na_2CO_3/NaHCO_3$. Sample pretreatment: 0.45 µm filtration. Sample: (1) Chloride, (2) Nitrate, (3) $[GdDTPA]^{2-}$, (4) Sulphate.

120x96mm (300 x 300 DPI)