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Journal of Chromatography A, 739 (1996) 307–315

JOURNAL OF
CHROMATOGRAPHY A

Ion chromatographic determination of chelating ligands based on the postcolumn formation of ternary fluorescent complexes

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Abstract

The formation of the lutetium, *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid, and 8-hydroxyquinoline-5-sulfonic acid (Lu–CDTA–HQS) fluorescent ternary complex is used to determine chelating ligands. The ligands are complexed with Lu^{3+} and separated on an anion-exchange column. The eluent mixes with 1 m*M* CDTA–HQS postcolumn reagent. CDTA displaces the analyte ligand from its lutetium complex to form the more stable Lu–CDTA complex, which further complexes with HQS to form the ternary fluorescent complex. The mechanism and kinetics of this postcolumn reaction are discussed. The scheme of sequential addition of CDTA–HQS at low pH and followed by NaOH enables detection of triethylenetetramine (Trien), tetraethylenepentamine (Tetren), nitrilotriacetic acid (NTA), ethylene glycol-bis(β-aminoethylether) N,N,N′,N′,-tetraacetic acid (EGTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepenta-acetic acid (DTPA) and CDTA. The ligands of NTA, EGTA, EDTA, DTPA, and CDTA were separated by ion chromatography and detected with postcolumn addition of CDTA–HQS (pH 2.8) and then NaOH. The detection limits obtained were $1\cdot10^{-7}$ *M* (ca. 1 ng) for NTA, $2.5\cdot10^{-8}$ *M* (ca. 0.5 ng) for EGTA, $2.5\cdot10^{-8}$ *M* (ca. 0.5 ng) for EDTA, $5\cdot10^{-8}$ *M* (ca. 1 ng) for DTPA, and $5\cdot10^{-8}$ *M* (ca. 1 ng) for CDTA. The response linear range for NTA was from 100 ng to 2.5 ng; for EGTA from 750 ng to 1 ng; for EDTA from 750 ng to 1 ng; for DTPA from 5000 ng to 1 ng; and for CDTA from 5000 ng to 2 ng. The existence of ten-fold excess alkaline earth metal and transition metal ions did not interfere with the determination of these chelating ligands after a metal-exchange sample pretreatment step was included in the procedure. The recovery rates were greater than 80%.

Keywords: Complexation; Chelating ligands; Derivatization, LC; Lutetium; Aminopolycarboxylic acids

1. Introduction

Aminopolycarboxylic acids are powerful chelating ligands and thus are widely used in industry, agriculture, and medicine. For instance, nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) are used in detergents while diethylenetriaminepentaacetic acid (DTPA) and EDTA are added to commercial fertilizers and used in the pulp and paper industry. The release of these chelating ligands to the environment changes the natural water conditions. These chelating ligands dissolve heavy metals in sediments [1] and thus alter the biochemical cycle of heavy metals in water [2,3]. Due to the environmental impact of these chelating ligands, a sensitive means of analysis is required.

Several analytical techniques exist for the determination of these chelating ligands. It has been shown that chromatography is the most suitable method for the low-level determination of these chelating ligands [4]. Gas chromatographic methods have been reported [5,6], but these methods require a prederivatization step which is time-consuming [7,8]. Liquid chromatography has been usually used to

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PII S0021-9673(96)00047-7

determine aminopolycarboxylic acids [7,9–19]. Indirect UV detection [17] and electrochemical detection [14–16] have been used as liquid chromatographic detectors. Typically, direct UV spectrophotometry of the Fe^{3+} or Cu^{2+} chelates is used to determine chelating ligands [7,10–12,18,19]. However, any UV-absorbing matrix component interferes with the analyses.

In our previous study, a sensitive and selective method for the determination of hexadentate ligands was developed using a fluorescence detector [9]. This detection technique is based on the postcolumn formation of a ternary complex between lutetium, 8-hydroxyquinoline-5-sulfonic acid (HQS), and the hexadentate aminopolycarboxylic acids. Ligands with dentation other than six do not form a ternary complex with lutetium and HQS, thus they do not yield a fluorescent response.

In this work, the postcolumn formation of a ternary complex is extended to the determination of other chelating ligands (e.g. NTA, DTPA, etc.) in addition to hexadentate ligands. The chelating ligands are separated as their 1:1 complex with Lu^{3+} After separation the eluate merges with a reagent of *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CDTA) and HQS. CDTA displaces the analyte ligand from lutetium to form a Lu–CDTA complex, which further forms a Lu–CDTA–HQS fluorescent ternary complex. The mechanism and kinetics for the displacement of an analyte and the formation of a ternary complex will be discussed in this manuscript. The application of ion chromatography for separation and determination of several aminopolycarboxylic acids using a CDTA–HQS postcolumn reagent system is shown in the following sections.

2. Experimental

2.1. Apparatus

The HPLC system consisted of a metal-free solvent delivery system (Model 625, Waters Associates, Milford, MA, USA), a sampling valve (Model 9125, Rheodyne, Berkeley, CA, USA) fit with a 50-μl loop. The column was a 150×4.6 mm C_{18} reversed-phase column packed with 5-μm ODS-2 particles (Phenomenex, Torrance, CA, USA). A fluorimetric detector (Model 470, Waters; excitation λ=360 nm; emission λ=500 nm) was used. The output signal from the detector was digitized with a CHROM-1AT (Keithley MetraByte) data acquisition board enhanced for faster data collection. The data acquisition and analysis was processed using Lab-Calc (Galactic, Salem, NH, USA) software on a 286-based microcomputer.

The postcolumn reagent was delivered by constant pressure pumping through application of compressed-gas (i.e., nitrogen or helium) pressure to a cylinder containing the reagent [20,21]. The pressure varied from 36 to 40 p.s.i. ($2.5\cdot10^5$–$2.8\cdot10^5$ Pa) for the experiments. The reagent flow-rate was measured for each experiment. Low-pressure tubing and fittings (Alltech Assoc., Deerfield, IL, USA) connected the postcolumn reagent delivery system to the effluent stream. The eluent from the HPLC pump (1.0 ml/min) merged with the postcolumn reagent (flow-rate was measured for each experiment) at the mixing tee (316 SS, 90° ports), flowed through a postcolumn reactor and then directly to the detector. The postcolumn reactor was 510 cm of tightly spiraled 0.5 mm I.D. knitted Teflon tubing (RXN 1000 Coil, Waters; 1000 μl volume).

2.2. Reagents and standards

All reagent solutions were prepared using deionized water (Nanopure Water System, Barnstead). Analytical-grade reagents were used throughout. Stock 0.01 *M* HQS and 0.01 *M* CDTA were prepared by dissolving HQS (Janssen Chimica) and CDTA (Janssen Chimica) in deionized water, respectively. The 1 *M* stock 2-amino-2-methyl-1-propanol (AMP) (J.T. Baker) buffer was prepared in deionized water and adjusted to pH 10 with acetic acid (BDH). The postcolumn reagents were prepared from the stock HQS, CDTA and buffer solutions. For the experiment of simultaneous addition of postcolumn reagent, the postcolumn reagent was 1 m*M* HQS, 1 m*M* CDTA in 0.1 *M* AMP at pH 10. For the experiment of sequential addition of postcolumn reagents, one reagent was 1 m*M* HQS and 1 m*M* CDTA at pH 2.8 adjusted by acetic acid, and the second was 1 *M* of sodium hydroxide (BDH).

The eluent consisted of tetrapropylammonium bromide (TPAB), potassium sulfate (K_2SO_4) and

acetonitrile (BDH). Stock 0.01 *M* TPAB and 0.01 *M* K_2SO_4 solutions were prepared by dissolving TPAB (98%, Aldrich) and K_2SO_4 (Fisher Scientific), respectively, in deionized water.

The 1 m*M* Lu^{3+} stock solution was prepared by dissolving lutetium chloride hexahydrate (99%, Aldrich) in deionized water. Stock 1 m*M* triethylenetetramine hydrate (Trien), tetraethylenepentamine (Tetren), NTA, ethylene glycol-bis(β-aminoethylether) N,N,N′,N′,-tetraacetic acid (EGTA), EDTA, DTPA and CDTA solutions were prepared by dissolving Trien (98%, Aldrich), Tetren (Sigma), NTA (Eastman), EGTA (97%, Sigma), DTPA (97%, Janssen Chimica) and CDTA (98%, Janssen Chimica), respectively, in deionized water. Lower concentrations were achieved by quantitatively diluting these stock solutions individually in volumetric Nalgeneware. All solutions were stored in Nalgene plastic bottles.

The Lu–ligands mixture was $1 \cdot 10^{-5}$ *M* NTA, EGTA, EDTA, DTPA, CDTA mixed with $1 \cdot 10^{-4}$ *M* Lu^{3+} solution. Excess Lu^{3+} was removed from the original mixture by passage through a 7×2 cm I.D. column of AG 50W-X8 cation-exchange resin (70–130 μm, in the sodium form, Bio-Rad). Lower concentrations were obtained by diluting the Lu^{3+}–ligand mixture solution in volumetric Nalgeneware. All samples, buffer and eluents were filtered through 0.45-μm cellulose nitrate membrane before use.

To determine the effect of metal ions, $1 \cdot 10^{-4}$ *M* of each metal ion was spiked into $1 \cdot 10^{-5}$ *M* solutions of EGTA, EDTA, DTPA, and CDTA. The solutions were adjusted to pH 2.5 before passage through a 5×2 cm I.D. column of AG 50W-X8 cation-exchange resin (70–130 μm, in the hydrogen form, Bio-Rad). These solutions were made at 1 m*M* Lu^{3+} by adding lutetium chloride. The mixtures were adjusted to pH 7 and passed through a 5×2 cm I.D. column of AG 50W-X8 cation-exchange resin (70–130 μm, in the sodium form, Bio-Rad).

2.3. Procedures

Initial experiments were performed in the flow injection analysis mode; i.e., no separation column was present. For experiments of addition of single postcolumn reagent, the sample was injected from the sampling valve and carried by the 1.0 ml/min deionized water from the HPLC pump which merged with 0.45 ml/min postcolumn reagent (i.e. 1 m*M* HQS and 1 m*M* CDTA in 100 m*M* AMP at pH 10). The mixture of sample and postcolumn reagent mixture passed through the reaction coil and then flowed through the sample cell of the detector. For experiments of sequential addition of postcolumn reagents, 50 μl sample was carried by the 1.0 ml/min deionized water from the HPLC pump which merged with the 0.3 ml/min reagent 1 (1 m*M* CDTA and 1 m*M* HQS, pH 2.8). The mixture passed through the postcolumn reactor and combined with the 0.3 ml/min reagent 2 (1 *M* NaOH), and then flowed directly to the sample cell of the detector. Peak area was used as a measurement of the fluorescence intensity.

For the separation of Lu–ligand complexes, a reversed-phase separation column (Prodigy 5 ODS-2, C_{18} 5 μm, 150×4.6 mm HPLC column, Phenomenex) was employed. The eluent was 1.0 ml/min 3 m*M* TPAB in 1 m*M* K_2SO_4 for 4 min; then concentrations of K_2SO_4 and acetonitrile increased linearly to 3 m*M* and 4% (v/v), respectively, over 3 min, meanwhile the concentration of TPAB decreased linearly to zero over the same time period. The first postcolumn reagent containing 1 m*M* HQS and 1 m*M* CDTA (pH adjusted to 2.8 by acetic acid) was added to the column effluent at 0.3 ml/min and flowed through the postcolumn reactor. The second postcolumn reagent containing 1 *M* NaOH merged at 0.3 ml/min with the effluent from the postcolumn reactor and passed through the sample cell of the detector. The final effluent was at pH 12.0. Peak areas were used for quantification.

3. Results and discussion

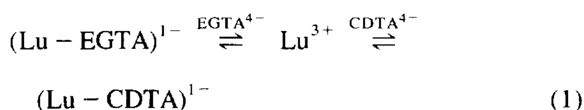
3.1. Theory

HQS like its parent compound (8-hydroxyquinoline; oxine) forms stable complexes with a wide variety of metal ions. Only a few metal ions form fluorescent complexes. The ion Lu^{3+} forms a fluorescent complex with HQS [22]. At the high pH needed to form the Lu–HQS complex, lutetium forms hydroxide complexes [23,24]. Formation of lutetium hydroxide can be prevented by addition of

chelating ligands such as CDTA. CDTA forms a more stable complex with lutetium than HQS or hydroxide [23–25]. Lutetium possesses eight coordination sites [26]. Thus lutetium complexes with the hexadentate ligand CDTA have two coordination sites available for further complexation. The bidentate ligand HQS adds to the Lu–CDTA to form the ternary Lu–CDTA–HQS complex [9]. This ternary complex is a sensitive and selective probe for determination of hexadentate ligands (e.g. EDTA, CDTA, and N-(hydroxyethyl)ethylenediamine triacetic acid (HEDTA).

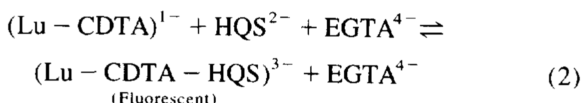
Other chelating ligands containing dentation other than six do not form a fluorescent ternary complex with Lu^{3+} and HQS [9]. For instance, the octadentate ligand EGTA forms a complex with lutetium which does not have any coordination sites free. Thus EGTA masks Lu^{3+} from HQS and the mixture of Lu^{3+}, EGTA and HQS is nonfluorescent.

If Lu–EGTA is added to a solution of CDTA, CDTA and EGTA compete with each other for complexation with Lu^{3+}.



$$(\mathrm{Lu-EGTA})^{1-} \overset{\mathrm{EGTA}^{4-}}{\rightleftharpoons} \mathrm{Lu}^{3+} \overset{\mathrm{CDTA}^{4-}}{\rightleftharpoons} (\mathrm{Lu-CDTA})^{1-} \quad (1)$$

The more stable Lu–CDTA complex is dominant in solution [25]. ($K'_{f,Lu-CDTA}/K'_{f,Lu-EGTA}$ is 900 at pH 3 and 28 at pH 10; K'_f is the conditional formation constant). Once the lutetium is complexed by CDTA only two coordination sites remain free. Both EGTA and HQS could occupy these sites as a bidentate ligand. Given the much greater concentration of HQS, it will dominate and so the fluorescent Lu–CDTA–HQS complex will be formed.



$$(\mathrm{Lu-CDTA})^{1-} + \mathrm{HQS}^{2-} + \mathrm{EGTA}^{4-} \rightleftharpoons \underset{\mathrm{(Fluorescent)}}{(\mathrm{Lu-CDTA-HQS})^{3-}} + \mathrm{EGTA}^{4-} \quad (2)$$

The intensity of the resultant fluorescence is proportional to the concentration of EGTA.

Other chelating ligands which form weaker complexes with Lu^{3+} than CDTA will also result in the formation of the fluorescent ternary Lu–CDTA–HQS complex. In this work these chelating ligands are separated as their lutetium complexes on a chromatographic column, and then monitored by the fluorescence response resulting from the post separation ligand exchange formation of Lu–CDTA–HQS.

3.2. Single postcolumn reagent

The chelating ligands Trien, Tetren, NTA, EGTA and DTPA are not hexadentate. Thus they do not directly form fluorescent ternary complexes with Lu–HQS [9]. Nonetheless their lutetium complexes can be determined using the CDTA–HQS reagent system, as discussed above. To demonstrate this experimentally the response observed for each ligand with the CDTA–HQS reagent was determined using flow injection analysis. Samples of $1 \cdot 10^{-5}$ *M* of each Lu–ligand (ligand = Trien, Tetren, NTA, EGTA, and DTPA here) were injected into a distilled water carrier stream using the 50-μl sample loop. This merged with the postcolumn reagent which was 1 m*M* CDTA and 1 m*M* HQS adjusted to pH 10 using 0.1 *M* AMP. A pH of 10 is used to ensure full deprotonation of HQS and maximum ternary complex stability. The intensity observed for each Lu–ligand complex was compared to the intensity for the same molar concentration of Lu^{3+}, and is shown in Table 1.

The lutetium complexes of Trien, Tetren, and NTA respond to this reagent but the lutetium complexes of EGTA and DTPA are not detectable. At pH 10, the conditional formation constant for Lu–DTPA is larger than that for Lu–CDTA ($K'_{f,Lu-DTPA}/K'_{f,Lu-CDTA} = 100$) and the conditional formation

Table 1
Relative intensity of samples upon single addition of CDTA–HQS

$1 \cdot 10^{-5}$ *M* sample	Relative intensity[a]
Lu	1.00
Lu–Trien	1.18
Lu–Tetren	0.97
Lu–NTA	0.80
Lu–EGTA	nd[b]
Lu–DTPA	nd

Conditions: mode = flow injection analysis; reaction coil = 510 cm of 0.5 mm I.D. tubing; carrier flow = 1.0 ml/min deionized water; reagent flow = 0.45 ml/min of 1 m*M* CDTA–HQS in 0.1 *M* AMP (pH 10).

[a] Relative intensity is defined as the intensity of sample divided by the intensity of Lu^{3+}.

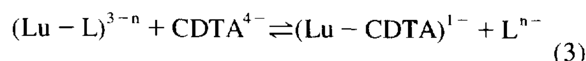
[b] nd = Not detected.

constant for EGTA is smaller than for CDTA ($K'_{f,Lu-EGTA}/K'_{f,Lu-CDTA}=0.036$) [25]. Thermodynamically, no response for DTPA is expected at pH 10. However, CDTA should displace EGTA from its lutetium complexes and form a Lu–CDTA complex which forms the fluorescent ternary complex with HQS. Previous study on metal–CDTA complex depicted that the decomplexation of metal–CDTA complex is a slow process at pH 10 [27]. Likewise, dissociation of Zn–EDTA is proven to be a slow step [28]. DTPA and EGTA is analogous to EDTA and CDTA. Kinetically, the decomplexation of DTPA and EGTA is slow at such high pH (i.e. pH 10). The reaction time in the postcolumn reactor is less than 1 min. This is not enough time for the Lu–DTPA or Lu–EGTA to decomplex to release Lu^{3+}. Therefore, taking both thermodynamic and kinetic factors into account, no significant response is expected for the injection of Lu–EGTA and Lu–DTPA complexes using a CDTA–HQS reagent at pH 10.

3.3. Sequential addition of postcolumn reagents

As discussed above, use of a single postcolumn reagent did not yield a response for Lu–EGTA and Lu–DTPA complexes. To circumvent the kinetics problem, sequential addition of reagents was employed. In this configuration the first postcolumn reagent (i.e., 1 m*M* CDTA and 1 m*M* HQS at pH 2.8 adjusted by acetic acid) is added to the stream carrying the sample. This mixture flows through the postcolumn reactor, combines with the second reagent (i.e., 1 *M* NaOH) and then flows to the detector. Using a flow injection mode, injections of $1\cdot10^{-5}$ *M* of each Lu–ligand complex were reacted in this fashion. Table 2 lists the intensities observed for the Lu–ligands relative to the response observed for the same molar concentration of Lu^{3+}.

All Lu–ligands tested responded to the sequential addition of CDTA–HQS and then NaOH. When Lu–ligand (Lu–L) complex is mixed with CDTA, the equilibrium is as below.



$$(Lu-L)^{3-n} + CDTA^{4-} \rightleftharpoons (Lu-CDTA)^{1-} + L^{n-} \quad (3)$$

Given the high stability of Lu–CDTA complexes Eq. (3) is thermodynamically favored to proceed to the right. However, the slow decomplexation step for some Lu–L complexes can hinder the formation of the Lu–CDTA complex, as was observed above. The use of low pH (i.e., pH 2.8) results in acid-catalyzed decomplexation of the Lu–L complexes, thus allowing Eq. 3 to equilibrate in a much shorter time period. The low pH also suppresses the formation of the ternary complex due to protonation of HQS, and thus results in the weak fluorescent intensity. Addition of the second reagent (NaOH) increases the solution pH. At such high pH, Lu–CDTA forms a stable fluorescent ternary complex with HQS.

Ideally using this postcolumn detection scheme all chelating ligands should respond with a sensitivity equal to that of uncomplexed Lu^{3+}. That is, Eq. 3 should proceed fully to the right. This is observed for all Lu–ligands studied except for Lu–DTPA (Table 2). This results from the similar conditional formation constants for Lu–DTPA and Lu–CDTA [25]. Thus some Lu^{3+} remains as the nonfluorescent Lu–DTPA complex. Increasing the concentration of CDTA in the reagent increases the response observed for DTPA. Use of a 100-fold excess of CDTA resulted in quantitative response for DTPA.

Thus sequential addition of CDTA–HQS reagent at low pH and then NaOH to increase the pH is an effective reagent for the determination of Lu–ligand complexes. These ligands are not limited to hexadentate ligands, but rather all chelating ligands respond. This technique offers a method to determine a variety of Lu–ligand complexes.

Table 2
Relative intensity of samples upon sequential addition of CDTA–HQS and NaOH

$1\cdot10^{-5}$ *M* sample	Relative intensity[a]
Lu	1.00
Lu–Trien	1.12
Lu–Tetren	1.00
Lu–NTA	0.98
Lu–EGTA	1.00
Lu–DTPA	0.66

Conditions: mode = flow injection analysis; reaction coil = 510 cm of 0.5 mm I.D. tubing; carrier flow = 1.0 ml/min deionized water; reagent flow = 0.3 ml/min of 1 m*M* CDTA–HQS (pH 2.8) and then 0.3 ml/min of 1 *M* NaOH. pH of final effluent, pH 12.0.
[a] Relative intensity is defined as the intensity of sample divided by the intensity of Lu^{3+}.

The ideal response intensity for each of the Lu-ligands is equal to that for the same concentration of Lu^{3+}. However, for the Lu–Trien in Table 1 and Table 2, the intensities measured are higher than that of Lu^{3+}. No explanation for this observation can been made at this stage.

3.4. Application of CDTA–HQS postcolumn reagent in ion chromatography

To determine the chelating ligands simultaneously, ion chromatography was employed. Chelating ligands were determined as their Lu^{3+} complexes. Lu^{3+} was added to the ligand sample to convert all ligands to their Lu–ligand complexes. EGTA, EDTA, DTPA, and CDTA carry a negative charge upon complexation with Lu^{3+} while Lu–NTA complex is neutral. Separations were carried out on a C_{18} column given anion-exchange capacity by equilibration with tetrapropylammonium bromide (TPAB). If the exact concentrations of ligands are unknown, excess Lu^{3+} must be added to the ligands solution to convert all ligands to their lutetium complexes. Fig. 1a is the chromatogram for a sample containing $1\cdot10^{-5}$ *M* NTA, EGTA, EDTA, DTPA, and CDTA spiked with $1\cdot10^{-4}$ *M* Lu^{3+}. Although the excess Lu^{3+} does not interfere with peaks of EGTA, EDTA, DTPA, and CDTA, the peak for NTA is overlapped by Lu^{3+} peak. As Lu–NTA is a neutral complex it is weakly retained under these conditions. To remove the excess Lu^{3+}, the sample was passed through a cation-exchange column (AG 50W-X8 resin) to retain the excess Lu^{3+}. The chelate complexes are stable and do not decomplex under these conditions. Fig. 1b is the chromatogram for the sample after removal of excess Lu^{3+} by the cation-exchange column. Comparison of Fig. 1b with the chromatogram, in which the exact amount of Lu^{3+} spiked into ligands solution, indicates quantitative (98–100%) recovery of EGTA, EDTA, DTPA and CDTA. A recovery of 75% was observed for the weaker NTA complex.

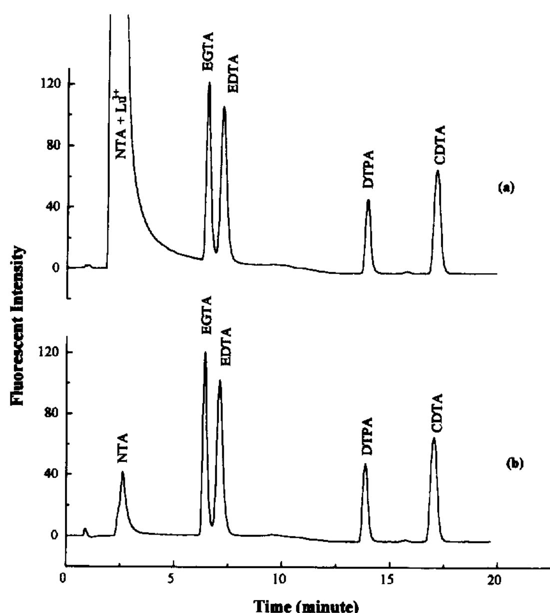


Fig. 1. Ion chromatographic separation of (a) $1\cdot10^{-4}$ *M* Lu^{3+} spiked into $1\cdot10^{-5}$ *M* each of NTA, EGTA, EDTA, DTPA, and CDTA; (b) same sample as in (a) passed through a cation-exchange column to clean up the excess Lu^{3+}. Conditions as described in the Experimental section.

Different concentrations of lutetium complexes of NTA, EGTA, EDTA, DTPA, and CDTA were determined. The linear ranges and detection limits observed for the tested ligands are shown in Table 3. For all ligands, linearity of calibration was excellent (r^2 greater than 0.99) with all intercepts within the 95% confidence interval and detection limits in the 10^{-8} *M* range. However, linear regression can mask a nonlinear response in many cases [29,30]. Therefore the calibration data was also analyzed using a sensitivity plot, as advocated by the American Society for Testing and Materials (ASTM) [31]. The sensitivity (S) is defined as:

$$S_i = I_i / C_i \quad (4)$$

where I_i and C_i are the fluorescent response and sample concentration at calibration point i, respectively. Within a linear response range, the sensitivities S_i will be constant, independent of the sample concentration. Drifting of the sensitivity with concentration illustrates curvature within the calibration data. Fig. 2 shows the plots of sensitivity versus concentration for the five ligands studied. Each of the ligands have been offset for greater clarity of presentation. The significant systematic deviation (<15%) is not observed for the plots of EGTA (△ in Fig. 2) and CDTA (□ in Fig. 2) within the range shown in Fig. 2. The sensitivities for EDTA (● in Fig. 2) remain constant at concen-

Table 3
Calibration curve constants[a] and the detection limits for the determination of chelating ligands with ion chromatography

Analyte	r^2	Slope ($\cdot 10^{-6}$)	y-Intercept	Dynamic range (*M*)	Detection limit[b] (*M*)
NTA	0.9984	1.90±0.03	−0.75±0.16	$2.5\cdot10^{-7}$–$1\cdot10^{-5}$	$1\cdot10^{-7}$
EGTA	0.9997	3.11±0.02	−0.13±0.08	$5\cdot10^{-8}$–$1\cdot10^{-5}$	$2.5\cdot10^{-8}$
EDTA	0.9999	3.83±0.01	−0.09±0.05	$5\cdot10^{-8}$–$1\cdot10^{-5}$	$2.5\cdot10^{-8}$
DTPA	0.9972	2.11±0.06	0.29±0.13	$1\cdot10^{-7}$–$5\cdot10^{-6}$	$5\cdot10^{-8}$
CDTA	0.9939	2.44±0.09	0.46±0.37	$1\cdot10^{-7}$–$1\cdot10^{-5}$	$5\cdot10^{-8}$

[a] Calibration curve is obtained from linear regression based on the experimental data.
[b] Detection limit is defined as three times the baseline noise. The unit here is mol/l and can be converted to mol or g based on the size of injection loop (50 μl) and the molecular mass of ligand.

trations larger than $1\cdot10^{-7}$ and the data point at $5\cdot10^{-8}$ *M* is still within 15% deviation range. Meanwhile, the sensitivities for DTPA (○ in Fig. 2) plot are all within the 15% deviation range except data points at $5\cdot10^{-8}$ *M* and $1\cdot10^{-5}$ *M* DTPA. The plot for NTA (+ in Fig. 2) has larger systematic drift than the other plots. This observation could be explained by the small stability constant for the NTA complex, which is easy to decomplex at low concentration. Therefore, the dynamic ranges shown in Table 3 are determined based on the constant sensitivities which are within 15% systematic deviation.

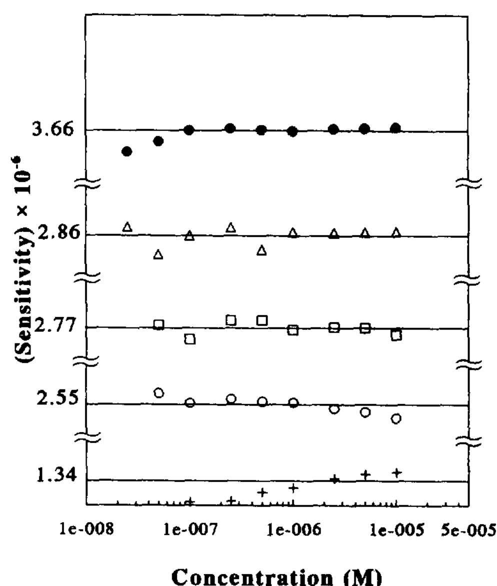


Fig. 2. Sensitivity of the determination of chelating ligands for concentration ranges described in Table 3. Conditions as described in the Experimental Section: ● = EDTA; △ = EGTA; □ = CDTA; ○ = DTPA; + = NTA.

The detection limits obtained by using this method compare well with detection limits observed for either DTPA using absorbance detection with the formation of Fe(III)–DTPA (ca. $1\cdot10^{-6}$ *M* [7]) or EDTA using absorbance detection with the formation of Cu(II)–EDTA (ca. $1\cdot10^{-7}$ *M* [15]) and Fe(III)–EDTA (ca. $1\cdot10^{-6}$ [7] and $3\cdot10^{-8}$ after preconcentrating a sample using a 100-μl sample loop [12]). No detection limit of EGTA using chromatography is reported according to our references searched. Compared with other ligands, NTA has a poor dynamic range and detection limit ($1\cdot10^{-7}$ *M*). This results from the poor separation of Lu–NTA and its less stable complexation. Nevertheless, the detection limit for NTA compares well with the detection limit observed by using an electrochemical detector in ion chromatography (ca. $2\cdot10^{-5}$ *M*) [14].

Lutetium complexes of Trien and Tetren are positive in charge. An attempt to separate the Lu^{3+} complexes of Trien and Tetren on a cation-exchange column was unsuccessful due to their decomplexation at low eluent pH. Use of higher pH eluents for Trien and Tetren was also unsuccessful, presumably due to retention on silanol groups.

To determine the chelating ligands in a real sample, the potential interference for the determination of chelating ligands by using CDTA–HQS postcolumn reagent is the formation of the other metal–ligand complexes which prevent the ligands from complexing with Lu^{3+}. A variety of metal ions were added to the ligands solution and the cation-exchange column can remove the majority of the metal ions at pH 2.5. The addition of an excess of

Table 4
Recovery of the detection for chelating ligands with the interference of other metal ions

Metal ions	Recovery[a] (%)			
	EGTA	EDTA	DTPA	CDTA
Ca^{2+}	85	100	91	100
Mg^{2+}	91	100	91	100
Cu^{2+}	96	95	88	100
Co^{2+}	96	95	80	100
Ni^{2+}	86	86	80	100
Zn^{2+}	100	94	91	98
Mn^{2+}	100	88	96	86
Fe^{3+}	88	98	91	83

Conditions: ion chromatographic separation of $1 \cdot 10^{-5}$ *M* ligands with postcolumn formation of Lu–CDTA–HQS fluorescent ternary complex. Details of sample preparation and separation are described in the Experimental section.
[a] Recovery is defined as the intensity of the individual peak for the sample with other metal interference divided by the intensity of the individual peak for the sample without other metal interference.

Lu^{3+} displaces the trace amount of other metal ions from their complexes and quantitatively forms the Lu–ligand complexes. Table 4 shows that the alkaline earth and transition metal ions do not interfere with the determination of EGTA, EDTA, DTPA, and CDTA ligands significantly. Quantitative (>80%) recovery of EGTA, EDTA, DTPA, CDTA indicates that the formation of Lu–CDTA–HQS ternary complex is a perspective method to the determination of chelating ligands by using the fluorescent detector in ion chromatography.

4. Conclusions

Formation of ternary complexes of lutetium in the presence of 8-hydroxyquinoline-5-sulfonic acid provides sensitive and selective detection of chelating ligands separated by ion chromatography. Fluorescent ternary complexes with Lu and HQS only form with hexadentate ligands such as CDTA. The applicability of this reagent is expanded to include ligands of other dentation by performing ligand exchange in the postcolumn reaction detector. Detection limits for chelating ligands such as EGTA, EDTA, DTPA, and CDTA are in the 10^{-8} *M* range with linear calibration curves up to 10^{-5} *M*. Other chelating ligands such as Trien and Tetren also respond, but satisfactory chromatography of these ligands could not be achieved.

Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council of Canada and by the University of Calgary.

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