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Separation of uranium(VI) and lanthanides by capillary electrophoresis using on-capillary complexation with arsenazo III

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Abstract

The viability of the separation of lanthanides and uranium(VI) in the form of strongly absorbing complexes with arsenazo III (AIII) was studied with the aim to increase the sensitivity of absorbance detection in determination of these metals by capillary electrophoresis (CE). Special attention was paid to the complexation equilibria in the background electrolyte (BGE). On-capillary complexation provided better peak shapes for lanthanides compared to pre-column complexation. While the BGE composition had very little effect on the peak shape of the kinetically inert uranium(VI) complex, it played a crucial role in the peak shapes of the kinetically labile lanthanide complexes. Addition of a second ligand competing with the metallochromic ligand AIII for the metal ions was found to be critical to achieve good peak shape. The nature and concentration of the competing complexing ligand added to the BGE, the pH, and the concentration of AIII were found to exert a strong influence on the separation selectivity, peak shapes and the detection sensitivity. Several carboxylic acids were compared as BGE competing ligands and citrate provided best selectivity and peak shapes. A citrate BGE at pH 4.7 and containing 0.1 mM AIII was used for the separation of uranium(VI) (350 000 theoretical plates) and La^{III} (63 000 theoretical plates) while, to separate most lanthanides and uranium(VI), a similar BGE with a lower (0.03 mM) AIII concentration was used. Using hydrostatic sampling (100 mm for 10 s) detection limits of 0.35 μ M (49 ppb) La^{III} and 25 μ M (60 ppb) UO₂ were obtained. Using on-capillary complexation, sample stacking was retained for injection times of up to at least 100 s (ca. 30-mm sample plug) without loss of peak shapes for lanthanides or recovery for La^{III}. When this process was used, the detection limit for La^{III} was reduced to about 5 ppb. Optimal properties of metallochromic ligands for separation and detection of metals by CE are discussed. © 1998 Elsevier Science B.V.

Keywords: Complexation; Background electrolyte composition; Uranium; Lanthanides; Arsenazo III

1. Introduction

Numerous examples of separations of both small anions and small cations by capillary electrophoresis (CE) have been reported, and these generally exhibit much superior separation efficiency and shorter analysis times compared to ion chromatography (IC), but with concentration sensitivity usually lower than

IC by an order of magnitude or more [1–3]. This is the case even when both techniques are compared using the same detection system, for example indirect photometric detection, which is universal and is consequently frequently used for non-absorbing ions in CE and IC [2]. The main reason for the reduced sensitivity of CE is the much shorter optical path length (usually by two orders of magnitude) of the on-capillary photometric detector in CE compared to a typical photometric detector used in IC.

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To increase the sensitivity of absorption detection, some analytes can be converted to species with higher absorptivity [4]. If non-absorbing ions can be derivatised to absorbing species, direct photometric detection can be used instead of indirect, which generally results in superior limits of detection [1,2].

In the area of metal determinations, this approach is utilised when metals are determined as highly absorbing complexes with metallochromic ligands. Numerous photometric methods [5] and liquid chromatography–IC methods with photometric detection [1] exist. In the past several years also CE and a related method, micellar electrokinetic chromatography (MEKC), have been increasingly applied to separations of metal ions in the form of complexes with metallochromic ligands [3,6]. The metallochromic ligands used have included 4-(2-pyridylazo)resorcinol (PAR) [7–11], porphyrin derivatives [12], 2,2'-dihydroxyazobenzene-5,5'-disulfonate (DHAB-DS) [13], 2-(5-nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol (Nitro-PAPS) [14], 2,2'-(1,8-dihydroxy-3,6-disulfonaphthylene-2,7-bisazo)-bisbenzenearsonic acid (arsenazo III) [9,15], 2-nitroso-1-naphthol-4-sulfonic acid (Nitroso-NSA) [16], 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol (Br-PAPS) [17], 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (PADAP) [18,19], (2-(4,5-dihydroxy-2,7-disulfo-3-naphthylazo)phenylarsonic acid), (arsenazo I) [20,21], and (3,6-bis(2-sulfophenylazo)-4,5-dihydroxy-2,7-naphthalenedisulfonic acid (sulfonazo III) [22]. However, despite the high separation power of CE, often only a few metals (typically one to five metals [7–12,15,16,18–21]) have been separated, even though most of these ligands form complexes with a broad range of metal ions. Therefore problems in the application of this approach can be anticipated and consequently more research is needed to identify the factors which govern the separation process.

One of the most challenging tasks is the separation of lanthanide metal ions. Separations of lanthanides by CE have been achieved utilising secondary equilibria with an auxiliary complexing ligand [3]. All 14 lanthanides were separated using partial complexation with 2-hydroxyisobutyric acid (HIBA) [23–31] or lactic acid [29,30] and using indirect photometric detection. Direct absorption detection in the UV range has been utilised in the complete

separation of 13 lanthanides as complexes with 1,2-diamino-cyclohexane-*N,N,N',N'*-tetraacetic acid (CDTA) [32,33] in the total complexation mode. Somewhat surprisingly, no successful applications of metallochromic ligands to the separation of lanthanides have been reported. It should be noted that all the lanthanide separations have been achieved with relatively small auxiliary ligands.

Probably the most sensitive photometric reagents for a large number of di- and higher valent metals including uranium, thorium and lanthanides is arsenazo III (abbreviated AIII) [5,34]. It has been used in CE for separation of a model mixture of several transition metals, lanthanum, cerium(III) and uranium(VI) [9], but peaks for lanthanum and cerium(III) were poorly shaped and co-migrating. AIII has been also used for the determination of uranium(VI) in waters [15], but the peak obtained was broad. Arsenazo III is a relatively large molecule, so that differences in electrophoretic mobility caused by complexation with different metal ions are unlikely to be significant and it is therefore questionable whether a complete separation of a number of similar metal ions can be achieved using the total complexation mode.

The aim of the present study was to determine whether partial complexation with AIII using a competing auxiliary ligand added together with the AIII into the background electrolyte (BGE) is a workable way to separate lanthanides, uranium(VI) and thorium(IV) by CE and to study the role of the complexation equilibria in the BGE in the CE separation.

2. Experimental

2.1. Reagents

Carbowax 20M (poly(ethylene glycol) compound with 2,2'-[1-methylethylidene]bis(4,1-phenyleneoxymethylene)]bisoxirane, CW20M) and arsenazo III (2,2'-(1,8-dihydroxy-3,6-disulfonaphthylene-2,7-bisazo)-bisbenzenearsonic acid) were purchased from Aldrich. The purity of AIII determined using the earlier reported CE method [35] was 79% (external standard method using 98% pure AIII, Aldrich). Standard solutions of metals were prepared from the

Table 1
Chemicals used for preparation of standard solutions of metal ions

Metal	Chemical	Grade ^a	Supplier
La ^{III}	Lanthanum oxide	AR	Light, Colnbrook, UK
Ce ^{III}	Cerium (III) nitrate hexahydrate	AR	Strem Chemicals Newburyport, MA, USA
Pr ^{III}	Praseodymium nitrate	AR	Light
Nd ^{III}	Neodymium oxide	AR	Light
Sm ^{III}	Samarium oxide	AR	Light
Eu ^{III}	Europium oxide	AR	Light
Gd ^{III}	Gadolinium oxide	AR	Light
Tb ^{III}	Terbium (metal)	99.9%	Rhone Poulenc, Paris, France
Dy ^{III}	Dysprosium oxide	AR	Light
Ho ^{III}	Holmium oxide	AR	Light
Er ^{III}	Erbium oxide	AR	Light
Tm ^{III}	Thulium (metal)	99.9%	Rhone Poulenc
Yb ^{III}	Ytterbium oxide	AR	Light
Lu ^{III}	Lutetium trichloride with 2 mol of tetrahydrofuran	R	Professor G.B. Deacon, Monash University, Melbourne, Australia [57]
Th ^{IV}	Thorium nitrate hexahydrate	AR	May & Baker, Dagenham, UK
U ^{IV}	Uranyl nitrate hexahydrate	AR	Ajax Chemicals, Sydney, Australia

^aAR, analytical-reagent grade.

chemicals listed in Table 1. All chemicals other than nitrates were first dissolved in concentrated nitric acid and the nitric acid concentration in all stock solutions of metals was finally adjusted to approx. 0.1 M. All other chemicals were of analytical reagent grade. Water was treated with a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus.

Before use the BGE was degassed by vacuum and filtered with a Millex-HA 0.45- μ m disc filter (Millipore, Bedford, MA, USA).

2.2. Instrumentation

The 75- μ m I.D. fused-silica (FS) separation capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA) and used in 0.480-m (0.400 m to detector) or 0.600-m lengths (0.520 m to detector). The FS capillaries were used untreated, unless stated otherwise.

The instrument used was a Quanta 4000 (Waters, Milford, MA, USA) interfaced to a Millennium data station (Waters). Instead of the original mercury lamp, an LED-based light source using a 654-nm ultra-bright LED (RS 564-015, RS Components, Victoria, Australia) was used as described earlier [20–22].

Injection was performed hydrostatically by elevating the sample at 100 mm for 5–100 s at the cathodic side of the capillary. At the anodic (detec-

tor) side a buffer reservoir of 4 ml volume was used. Instead of the standard 20-ml buffer reservoir at the cathodic side, a plastic sample vial of approximately 0.6 ml was installed inside of the standard 20 ml container. The separation voltage was –30 kV.

3. Results and discussion

3.1. General approach

The majority of the previously published separations of metals as complexes with metallochromic ligands have used pre-capillary complexation as the preferred method of complex formation [3], with on-capillary complexation being used in only a few cases, such as with PAR [10], arsenazo I [20,21] or sulfonazo III [22]. Pre-capillary formation is preferable when the metal complexes are stable and often also kinetically inert. In these cases the metal ions are usually complexed totally. For totally complexed metal ions, the complexation equilibrium cannot be utilised as a source of separation selectivity by manipulating the effective mobility through variation of the degree of complexation of the metal ion. Therefore other approaches to the manipulation of separation selectivity have to be devised [3]. These approaches can include the formation of mixed hydroxocomplexes [33], expression of the metal

character into the pK_a of an acidic group of the ligand not involved in metal complexation (such as *p*-OH group of PAR) [8], or ion-pair and ion-exchange interactions [13].

It has been shown in our previous reports on separations of alkaline earth metal ions using complexation with arsenazo I [20,21] or sulfonazo III [22] that complexation equilibria can be a major source of separation selectivity by using partial complexation of the metal ion. However, since the stability constants of complexes of lanthanides with AIII are high ($\log \beta_{ML} = 7.9\text{--}7.2$ for La^{III} to Lu^{III} [36]), the new approach taken in this work was to utilise competing complexation equilibria with an additional ligand in the BGE that would decrease the conditional stability constants for the complexes with the metallochromic ligand (AIII). Such a decrease in the degree of complexation with the metallochromic ligand from total ($\alpha = 1$) to partial ($\alpha < 1$) can offer the complexation equilibria as a source of separation selectivity. Assuming different mobilities for AIII and competing ligand complexes with a metal ion, varying the ratio of the metal complexed with each ligand will affect selectivity. Additionally, the competing ligand should exhibit rapid complexation kinetics, as is the case for anions of small carboxylic acids, such as citrate [25]. Regarding the degree of complexation with AIII, a substantial portion of the separated metal ions should still remain complexed with the AIII in order to enable sensitive direct detection of the AIII metal complexes.

3.2. Choice of the capillary wall chemistry

A comparison of several different capillary wall chemistries including bare fused-silica and several modified fused-silica capillaries showed that best results in terms of peak shape and reproducibility were achieved using a bare fused-silica capillary (any pre-treatment with hydroxide has to be avoided) which had been additionally pre-conditioned (dynamically coated) by Carbowax 20M. Such a capillary was used throughout this study.

3.3. Kinetic stability of the metal complexes of arsenazo III

There is an obvious difference in the kinetic

stability of the AIII complexes of U^{VI} and La^{III} . When injecting complexes (formed pre-capillary) into a BGE not containing any AIII, the U^{VI} complex was detected but the La^{III} did not give any peak of the complex. With AIII in the BGE both U^{VI} and La^{III} could be detected. Consequently, uranium(VI) could be determined after pre-capillary complexation with AIII (as reported by Colburn et al. [15]), but the La^{III} complex is not kinetically inert and therefore AIII must be present in the BGE to stabilise the complex. As a consequence of the kinetic inertness of the U^{VI} AIII complex, the complexation equilibrium between the metal and ligand did not exert much influence on the migration time of U^{VI} (see Section 3.7) and therefore variation of the BGE composition could not be used as a source of separation selectivity for U^{VI} . Exactly the opposite was true for the kinetically labile lanthanide–AIII complexes which offered a tool for governing the separation selectivity of the lanthanides and a thus a mechanism for their separation.

3.4. On-capillary complexation

The possibility of application of on-capillary complexation is limited to complexes with relatively fast complex formation kinetics [3], which obviously is so in cases when reasonable peak shape and separation efficiency are achieved using on-capillary complexation. Pre-capillary and on-capillary complexation were compared and the results are shown in Fig. 1. An interesting phenomenon was observed for pre-capillary complexation (Fig. 1a) in that a very broad peak was obtained for La^{III} when a sample solution of $10 \mu\text{M}$ Th^{IV} , U^{VI} and La^{III} in BGE containing 1 mM AIII was injected into a BGE containing 0.1 mM AIII. Although the concentration of citrate in the BGE was two orders of magnitude higher than that of AIII, calculations show that under the conditions used only about half of the lanthanum is complexed by citrate. As the complexation kinetics for citrate are likely to be rapid [25], the citrate concentration cannot explain the observed peak broadening. However, this peak broadening for injections of metal ions containing a higher AIII concentration than that present in the BGE may be explained by the formation of La –AIII complexes of

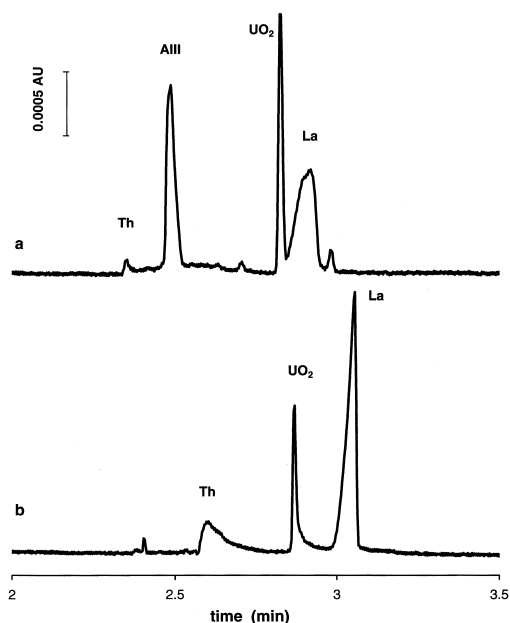


Fig. 1. Electropherograms of separations of Th^{IV}, U^{VI} and La^{III} when the sample was injected as (a) a solution in BGE with 0.1 mM AIII added, and (b) in 10 mM HNO₃. Capillary, FS 0.480 × 0.400 m, pre-run dynamically modified with CW20M (flushed before every run with 1% CW20M for 1 min, then with water for 0.5 min and BGE for 1 min); BGE, 0.10 mM AIII in 10 mM citric acid and 15 mM Tris (pH 4.7); separation voltage, -30 kV (39 μA); temperature, 25°C; detection, LED 654 nm; injection, hydrostatic (5 s, 100 mm) of a standard solution containing 10 μM of each metal. For other conditions see Section 2.

higher stoichiometry (such as 1:2) in the sample [5,34], which may not dissociate quickly enough after injection into a BGE with a lower AIII concentration in which 1:1 complexes prevail. Assuming different mobilities for the different complexes, such slow reactions after the injection would necessarily lead to deterioration of the peak shape. On the other hand, good peak shapes for both UO₂ and La^{III} were observed when injecting free metal ions in 10 mM HNO₃ (Fig. 1b). This suggested that the kinetics of complex formation for these metals were sufficiently fast, even for the U^{VI}-AIII complex. This observation is in agreement with other studies showing fast complex formation kinetics and slow complex dissociation for the U^{VI}-AIII complex [37–39]. Therefore the composition of the injected solution was of crucial importance and consequently for the remain-

der of the study metal solutions in diluted nitric acid (usually 10 mM) were injected.

3.5. Peak shapes and the role of the kinetics of complexation equilibria

Using BGE of 0.1 mM AIII and 15 mM citrate at pH 4.7, the separation shown in Fig. 1b was obtained. The efficiency was 350 000 theoretical plates for uranium(VI) and 63 000 theoretical plates for lanthanum. Thorium always formed a broad peak unsuitable for quantification, and so the purpose of its separation from U^{VI} and La^{III} was to ensure their selective determination. The separation efficiency for La^{III} was significantly lower than for uranium(VI) and the peak shape was 'fronted'. This cannot be explained by sorption effects onto the capillary wall (which usually cause tailing) nor by non-matching of the mobility of the BGE co-ion (the effective mobility of citrate at pH 4.7 is approx. $-62.1 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, and for the La^{III} complex $-34.4 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, i.e. the non-matching co-ion mobility should cause a tailed peak). However, the observed peak shape can be attributed to the complexation equilibria of La^{III} if the dissociation of the La^{III}-AIII complex is slow. Slow dissociation of AIII complexes with uranium(VI) has been shown in detailed spectrophotometric studies [37–39], but no kinetic studies for La^{III} were located. However, if slow dissociation kinetics for La^{III} did apply, any undissociated complex having a higher absolute value of anionic mobility than the equilibrium zone would migrate out of this zone in the direction towards the detector (anode) and so cause additional zone broadening and the observation of a fronted peak.

This effect of kinetics of complexation equilibria which limit the attainment of equilibrium during migration in the electric field, and consequently causing additional zone broadening, is called electrodiffusion, and was described well before the introduction of CE [40–45]. While both theory of kinetic effects and examples can be found in the literature concerning the historically older electromigration separation methods, such as paper electrophoresis or isotachopheresis [46–49], to our knowledge there are very few works in the field of CE of metal ions which discuss kinetic effects. Gebauer

and Boček [50] studied the migration of 1,2-diamino-cyclohexane-*N,N,N',N'*-tetraacetic acid (CDTA) complexes of Ca^{II} , La^{III} and Ho^{III} and observed bleeding of the zones under isotachophoretic conditions and peak broadening under zone electrophoretic conditions, with both effects being explained by non-instantaneous complexation equilibria. Also, Swaile and Sepaniak [51] reported that the peak shapes of Ca^{II} , Mg^{II} and Zn^{II} , when separated as complexes with 8-hydroxyquinoline-5-sulfonic acid (HQSA), depended strongly on the BGE parameters (ligand concentration, pH). The observed poor peak shapes were explained by the existence of several forms of the analyte within the migrating zone (namely the free metal, 1:1 and 1:2 complexes) and a slow rate of exchange between these forms. Throughout the remainder of the present study, efforts were therefore made to minimise the electrodiffusion peak broadening.

3.6. Choice of the BGE buffer

If the BGE buffer should compete with the metallochromic ligand (AIII) for the analyte metal ions as explained in Section 3.1, the conditional stability constants of the complexes should be comparable. This is the case for citrate (see Table 2; the complexation to second (ML_2) or higher degrees will be negligible in the acidic pH range of BGE used in this work).

Indeed, addition to the BGE of a suitable ligand was found to have a crucial influence on both the peak shapes and the selectivity of the separation.

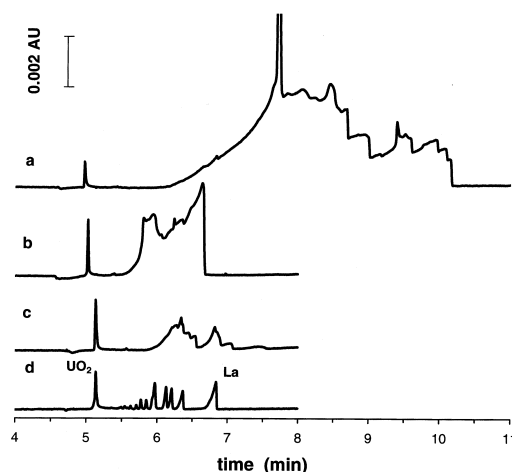


Fig. 2. Electropherogram of a separation of U^{VI} and 14 lanthanides in various BGEs: (a) 15 mM tartaric acid, 22 mM Tris, pH 4.1; (b) 30 mM acetic acid, 20 mM Tris, pH 4.4; (c) 15 mM HIBA, 10 mM Tris, pH 4.1; and (d) 15 mM citric acid, 15 mM Tris, pH 4.3; all BGEs also contained 0.025 mM AIII. Capillary, FS 0.6000×0.520 m, pre-run dynamically modified with CW20M; injection, hydrostatic (10 s, 100 mm) of a standard solution containing 10 μM of each metal. Other conditions as in Fig. 1.

Several mono-, di- and tricarboxylic acids (formic, acetic, glycolic, HIBA, tartaric, citric) were studied as BGE ligands (Fig. 2). It should be noted that the pH of the BGEs tested was in a narrow range of 4.1–4.4, indicating that the major differences observed were caused by the type of ligand used. The concentration of ligand was also held constant (15 mM), and only acetate was used at a higher concentration (30 mM) since the acetate complexes exhibit the lowest stability constants of those ligands compared in Table 2. In all BGEs Tris was employed

Table 2

Dissociation constants of the ligands (AIII, citrate, tartrate, HIBA, acetate) and stability constants for some lanthanides with the ligands as ML complexes [36]

Metal ion or H^+	Logarithms of stability constants (for ML) or ligand dissociation constant $\text{p}K_{\text{a}}$				
	AIII	Citrate	Tartrate	HIBA	Acetate
H^+	2.6 ($\text{p}K_{\text{a}5}$)	3.14 ($\text{p}K_{\text{a}1}$)	2.98 ($\text{p}K_{\text{a}1}$)	4.72 ($\text{p}K_{\text{a}1}$)	4.75 ($\text{p}K_{\text{a}1}$)
H^+	4.29 ($\text{p}K_{\text{a}4}$)	4.77 ($\text{p}K_{\text{a}2}$)	4.34 ($\text{p}K_{\text{a}2}$)	—	—
H^{3+}	6.7 ($\text{p}K_{\text{a}5}$)	6.39 ($\text{p}K_{\text{a}3}$)	—	—	—
La^{3+}	7.85	7.1	2.89	2.62	2.02
Nd^{3+}	7.79	7.7	3.45	2.88	2.22
Gd^{3+}	7.73	7.72	3.32	3.08	2.16
Yb^{3+}	7.23	8.05	3.48	3.64	2.03
Lu^{3+}	7.21	8.07	3.76	3.67	2.05

as the counter-cation because it resulted in a lower separation current than when sodium was used. As illustrated in Fig. 2, only citrate provided good peak shapes. This correlates with the highest stability constants of lanthanide complexes for citrate among all the carboxylic acids tested (Table 2). That suggests that competition between the AIII and citrate may be responsible for the improved separation. Species distribution diagrams calculated for lanthanide metal ions in solutions of citrate and AIII as a function of pH show that such competition was indeed probable (Fig. 3). As the ratio of concentrations $[AIII]/[citrate]$ is the major factor influencing the relative amounts of the AIII and citrate complexes formed, and the range of concentrations of AIII at which partial complexation is achieved is quite low (see Section 3.7), the highest possible citrate concentration in the BGE is the optimal concentration. After consideration of the separation current, a final concentration of 15 mM citrate was used for the remainder of the study. To better understand the role of the BGE ligand, the influence of AIII concentration in the citrate BGE and the influence of BGE pH on the peak shapes and separation selectivity were investigated.

3.7. Effect of concentration of arsenazo III in the BGE

It can be expected that at an AIII concentration when most of the metal ions are complexed ($\alpha_M \rightarrow 0$) the separation selectivity should be poor because AIII is a large ligand (M_r 776.4) and the influence of any change in the ionic radius of the complexed metal would be relatively small. In accordance with the above prediction, co-migration of the lanthanides was observed at the relatively high AIII concentration of 0.2 mM in the BGE (Fig. 4a). Another mechanism of projecting a property of the metal ion into the mobility of the complex is through influencing the pK_a of a dissociable $-OH$ group of the ligand not taking part in the metal ion complexation, and consequently the charge of the complex. This approach has been suggested for PAR [8] using the $p-OH$ group but cannot be utilised here as the phenolic groups of AIII dissociate first in the alkaline region [5].

An alternative possibility for influencing the effec-

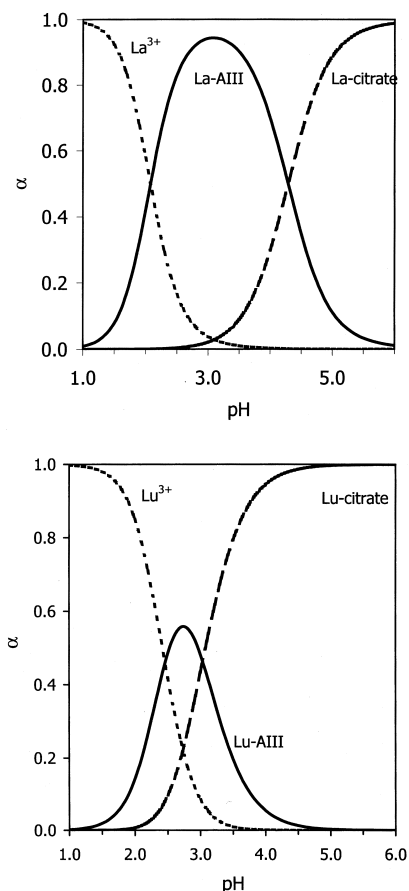


Fig. 3. α values for Me^{3+} , Me -AIII and Me -Cit systems for $Me=La^{III}$ and Lu^{III} , calculated from stability constants as in [36]. Complex equilibria were calculated based on the following reactions and equations: $La^{3+} + Hcit^{2-} \rightleftharpoons LaCit + H^+$; $La^{3+} + H_5AIII^{3-} \rightleftharpoons LaH_4AIII^{-} + H^+$; $\alpha_{Me} = 1/\text{denom}$; $\alpha_{MeCit} = K_{MeCit}[Cit^{3-}]/\text{denom}$; $\alpha_{MeAIII} = K_{MeAIII}[H_4AIII^{4-}]/\text{denom}$; $\text{denom} = 1 + K_{MeCit}[Cit^{3-}] + K_{MeAIII}[H_4AIII^{4-}]$, where H_3Cit =citric acid, H_8AIII =arsenazo III, α values are the fractions of metal ion present in the indicated form, K values are stability constants, and symbols of species given in square brackets are molar concentrations of those species.

tive mobility of an analyte is by utilising secondary equilibria in the partial complexation mode [3], which has been well documented in the use of HIBA or lactic acid for lanthanide separations [23–31]. Fig. 4 shows that the concentration of AIII in the BGE has a strong influence on (i) the separation selectivity, (ii) the peak shapes, and (iii) the detection sensitivity.

(i) With decreasing AIII concentration in the BGE,

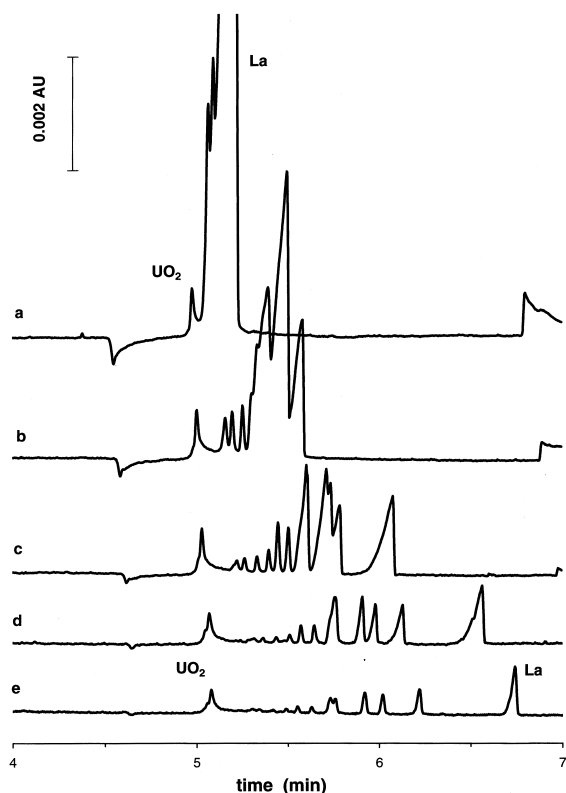


Fig. 4. Electropherogram of a separation of U^{VI} and 14 lanthanides in BGEs with varying AIII concentration: (a) 0.20 mM, (b) 0.10 mM, (c) 0.050 mM, (d) 0.025 mM, and (e) 0.0125 mM, in a BGE of 15 mM citric acid, 15 mM Tris, pH 4.3. Other conditions as in Fig. 2.

the migration time of the La^{III} peak increases, i.e. the absolute value of the anionic electrophoretic mobility decreases (Fig. 4): this corresponds to decreased formation of the negatively charged La^{III} -AIII complex and increased concentrations of a highly mobile positively charged metal cation, and also increased formation of neutral complexes with the competing ligand (citrate). Electrophoretic mobilities among the lanthanides also decrease in such a way that uranium(VI) and La^{III} form a 'window' inside which all of the lanthanides migrate. Most of the metal ions are separated at 0.0125 mM AIII (Fig. 4e). The concentration of AIII can be increased provided the citrate concentration is increased proportionally to keep the same distribution of metal ions between the two possible complexes. However, concentrations of citrate higher than approx. 25 mM could not be used

as a result of somewhat increased baseline noise probably due to higher current.

(ii) An essential condition for the employment of the partial complexation mode is that the equilibria involved should be sufficiently fast that additional peak broadening does not occur [3]. This problem occurs to a varying degree for the complexation with AIII, as shown by the observed broadening of the peaks in Fig. 4. The peak shapes of the lanthanides improve with decreasing AIII concentration (Fig. 4a–e), and these changes can be explained by competing complex equilibria of the separated metals with both the AIII and the citrate present in the BGE. The slow kinetics of the complexation equilibria with AIII are likely to cause the peak broadening evident at the higher AIII concentrations, while the kinetics of the complex equilibria with citrate are generally fast enough to participate in CE separations [25,52–54]. As discussed above (see Section 3.5) this suggests slow dissociation of the AIII complexes. Further evidence for the positive effect of citrate on the AIII–metal complex formation/dissociation kinetics can be found from the comparison of a separation under similar conditions as in Fig. 4 but with acetate, HIBA and tartrate BGEs (shown in Fig. 2), where all the peaks except for U^{VI} are excessively broad. Citrate forms generally much stronger complexes than acetate (Table 2), which means that the conditional stability constants for the AIII–metal complexes in the citrate buffer were lower than for the other BGEs.

(iii) With decreasing AIII concentration in the BGE the detection sensitivity decreases. This suggests that the proportion of the metal ions existing as AIII complexes decreases markedly and is in agreement with the strong correlation between the calculated α_{ML} values and relative detection sensitivity (expressed as migration time–normalised peak areas) shown in Fig. 5. A continuous decrease of relative detection sensitivity and conditional stability constant from La^{III} to Lu^{III} is evident.

3.8. pH of BGE

A further factor having a crucial role is the pH of the BGE. As shown earlier, the pH of the BGE influences the conditional stability constants of the metal complexes with both AIII and citrate. Sepa-

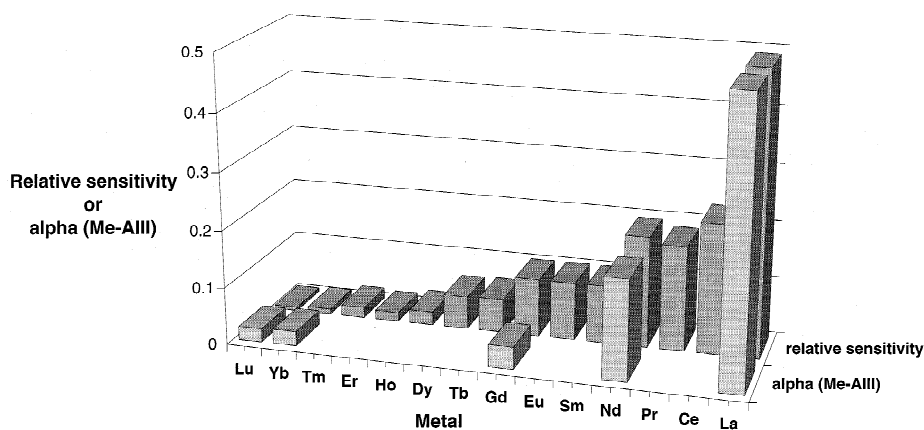


Fig. 5. Correlation of calculated α_{ML} values (for 0.025 mM AIII in BGE pH 4.3) of lanthanide–AIII complexes (Fig. 3) with relative detection sensitivity.

rations of U^{VI} , La^{III} and 10 lanthanides in a citrate BGE containing 0.025 mM AIII, at pH values in the range 4.0–5.0, are shown in Fig. 6. With decreasing pH the ‘window’ between the peaks of U^{VI} and La^{III} becomes wider and selectivity of the separation increases, but at the same time the peaks for the

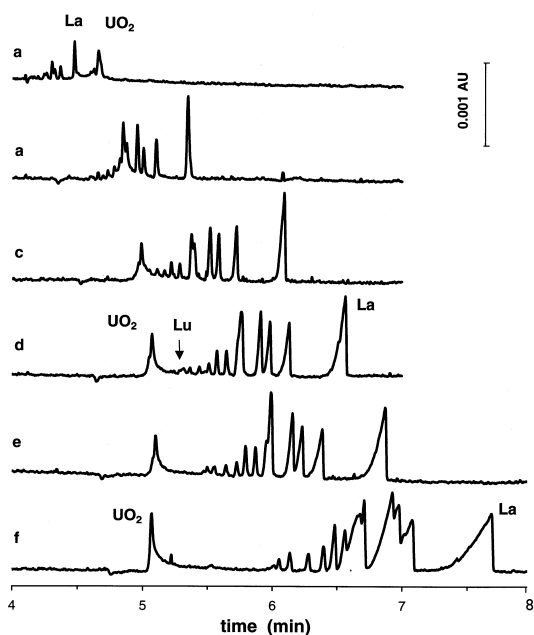


Fig. 6. Electropherogram of a separation of U^{VI} and 14 lanthanides in BGEs with varying pH: (a) 5.0, (b) 4.6, (c) 4.4, (d) 4.3, (e) 4.2 and (f) 4.0, in a BGE of 0.025 mM AIII in 15 mM citric acid and Tris up to pH. Other conditions as in Fig. 2.

lanthanides become broader. It can also be noted that at higher pH the detection sensitivity decreased markedly. This can be explained by competing equilibria of the metal ions with the AIII and citrate, with the conditional stability constants of citrate complexes increasing at higher pH, as confirmed by calculation of species distribution diagrams for La^{III} and Lu^{III} with AIII and citrate (Fig. 3). As the sensitivity and selectivity are optimal at the lower end of the pH interval, and the peak shape at the higher end, a compromise has to be found. As evident from Fig. 7c, separation of the maximum number of metal ions without excessive peak broadening was achieved at pH 4.3.

3.9. Optimised separation

The optimised separation of uranium(VI) and 14 lanthanides is shown in Fig. 7. Although Gd^{III} , Eu^{III} and Sm^{III} , as well as Yb^{III} and Lu^{III} , comigrated and the detection sensitivity for the last three lanthanides (Tm^{III} , Yb^{III} , Lu^{III}) was low, the remaining eight lanthanides (La^{III} , Ce^{III} , Pr^{III} , Nd^{III} , Tb^{III} , Dy^{III} , Ho^{III} , Er^{III}) as well as U^{VI} were baseline separated in under 7 min. As expected, the detection sensitivity decreased for lanthanides from La^{III} to Lu^{III} , according to the decreasing proportions of the AIII complex (α_{ML}) in the same order (Fig. 5). The limits of detection were 25 μM (60 ppb) for UO_2 and 0.35 μM (49 ppb) for La^{III} .

Finally, this separation was reproduced for a BGE

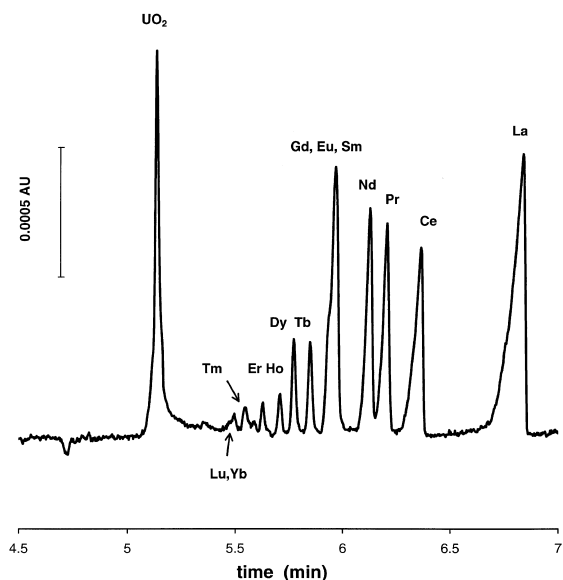


Fig. 7. Electropherogram of the optimised separation of U^{VI} and 14 lanthanides. BGE, 0.025 mM AIII in 15 mM citric acid and 20 mM Tris (pH 4.3); separation voltage, -30 kV ($30 \mu A$), injection of a standard solution containing $10 \mu M$ of each metal (except $20 \mu M$ for Tm^{III} , Yb^{III} and Lu^{III}) in 10 mM HNO_3 . Other conditions as in Fig. 2.

containing a 98% pure AIII (used for assay determination, see Section 2) and an identical separation was obtained when the AIII concentration in the BGE was adjusted in accordance with the determined purity of the AIII. This means that the influence of ligand purity on the separation reported recently for the metallochromic ligand arsenazo I [21] does not apply in this case.

3.10. Sample stacking

With on-capillary complexation, stacking based on the opposite mobilities of the injected aquated metal cations (positively charged) and the formed (negatively charged) complexes can be utilised [3,55,56], so that it is likely that higher sample volumes can be injected hydrostatically without the introduction of peak broadening effects. Indeed, hydrostatic injections for 10 and 100 s (Fig. 8) showed little difference in the separation, with only a shift in the migration times as a result of the injection of a large

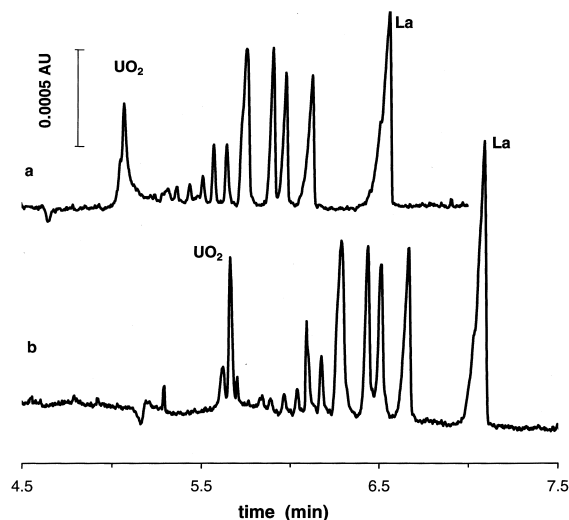


Fig. 8. Electropherograms of separations of U^{VI} and 14 lanthanides using a 10-s injection of a standard solution containing $10 \mu M$ of each metal (except $20 \mu M$ for Tm^{III} , Yb^{III} and Lu^{III}) and a 100-s injection of that standard solution $10\times$ diluted. Other conditions as in Fig. 7.

sample plug. The recovery for La^{III} in the 100-s injection was 106% (compared to the 10-s injection) and the limit of detection for La^{III} was decreased by a factor of 10 (ca. 5 ppb La). Uranium(VI) formed a split peak upon application of sample stacking, which may have been the result of the kinetic stability of U^{VI} complexes and further studies would be necessary to achieve application of sample stacking with on-capillary complexation for U^{VI} .

4. Conclusions

The following conclusions can be drawn with regard to (i) separation selectivity, (ii) separation efficiency and (iii) sensitivity of direct detection:

(i) Even a large ligand such as AIII can provide good separation selectivity towards a group of similar metals, such as the lanthanides, when secondary complexation equilibria are employed as the source of separation selectivity.

(ii) This is possible only if sufficiently fast kinetics of the complexation equilibria can be achieved, which in turn depends on the nature of the

metal ion and the ligand. Introduction of a competing ligand that itself exhibits fast kinetics of complexation equilibria can bring an improvement in the separation. It seems reasonable to anticipate that formation of only one type of complex (such as ML) is optimal for each metal ion analyte to avoid the possibility of slow kinetics of transition between different types of complexes.

(iii) Compared to complexation in the total complexation mode, the sensitivity of direct detection in the partial complexation mode can be expected to decrease in accordance with the proportion of metal ion present as the absorbing complex (i.e. α_{ML}).

Since the optimal conditions for each of the above criteria differ, a compromise has to be found when selecting the composition of the BGE. More studies including other metallochromic ligands would be necessary to explore the general viability of the partial complexation approach for metallochromic ligands.

With regard to properties of an optimal metallochromic ligand, the ligand should provide sensitive detection and broad selectivity (i.e. reacting with as many metal ions as possible) and should also either:

(i) form a single, stable complex with each of the separated metals when the total complexation mode is applied, which should allow projection of the properties of the metal ion into the electrophoretic mobility of the complex as the source of separation selectivity; or

(ii) should exhibit sufficiently fast kinetics of the complexation equilibria, and preferably form complexes to the first step only (ML), when the partial complexation mode is applied, in which case the complexation equilibria serve as the source of separation selectivity.

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