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Journal of Chromatography A, 796 (1998) 385–395

JOURNAL OF CHROMATOGRAPHY A

Simultaneous analysis of inorganic and organic lead, mercury and selenium by capillary electrophoresis with nitrilotriacetic acid as derivatization agent

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Received 22 April 1997; received in revised form 30 September 1997; accepted 30 September 1997

Abstract

A capillary electrophoresis (CE) method for the simultaneous speciation of three elements in organic and inorganic species was developed. Nitrilotriacetic acid (NTA) was used as the off-column derivatization agent to form UV-absorbing complexes with the analytes. It was also added to the running electrolyte for the separation and detection. Besides, micellar electrokinetic chromatography (MEKC) was performed with sodium dodecyl sulfate (SDS) for the adjustment of electrophoretic mobility. A theoretical model, with the consideration of pH, SDS and NTA in the electrolyte buffer, was designed to describe the migration behavior of the analytes. Good agreement was obtained when the model was used to explain the experimental results. The aspect of improvement in detection was also evaluated, and SDS was found capable of enhancing the detection sensitivity for the organometallic compounds. Further improvement of determination was achieved by amplified field sample injection. It permitted a 40 to 600 fold on-line enrichment for five analytes. Detection limits down to 0.2 ng/ml were obtained. © 1998 Elsevier Science B.V.

Keywords: Complexation; Derivatization, electrophoresis; Injection methods; Migration behaviour; Lead; Mercury; Selenium; Nitrilotriacetic acid; Metal cations; Organolead compounds; Oragnomercury compounds; Organoselenium compounds

1. Introduction

The speciation of elements has received much attention owing to their use in the agriculture, maritime, and other industries, as well as their occurrence in the environment, and their toxic properties [1]. As is known, elements released into the environment can be present as different species, including inorganic and organic forms or both, and these can exhibit different physicochemical properties and toxicity [1]. For example, inorganic and organic mercury compounds were found to co-exist in water and body tissues of some creatures [1]. In order to acquire comprehensive knowledge about the presence of an element in the environment, its metabolic process and to evaluate its influence, it is important to develop methods for simultaneously determining elements as different inorganic and organic species.

A variety of methods have been developed for this purpose. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) combined with mass spectrometry (MS), atomic absorption spectrometers (AAS) inductively coupled plasma

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PII S0021-9673(97)01012-1

(ICP) are commonly used approaches [2–4]. In recent years, capillary electrophoresis (CE) has made much progress in this field. Methods focusing on the simultaneous speciation of multielements in their organometallic compounds are occasionally available in CE [5–7]. Ng et al. [7] reported the direct UV detection and determination of organoselenium and organolead compounds. Micellar electrokinetic chromatography (MEKC) was conducted with β-cyclodextrin as the running electrolyte additive for separation. On the other hand, indirect detection is a viable and effective approach used for compounds without UV absorbing groups. Han et al. [8] studied the speciation of three organotin compounds. Pyridine was added to the running electrolyte as background electrolyte for indirect detection. Cetyltrimethylammonium bromide (CTAB) was also used to reverse the electroosmotic flow (EOF). It should be noted that the background electrolyte should have a similar electrophoretic mobility with most of the analytes to obtain symmetrical peaks in the indirect detection mode. Sometimes, the search for a suitable background electrolyte may be time-consuming. Other methods for the determination of organometallic compounds included the derivatization of analytes before the electrophoretic procedure. More recently, quantitative analysis of inorganic and organic arsenic anions was reported by Albert et al. [9,10]. Large-volume stacking injection was applied for the on-line concentration of the analytes. About a 50-fold improvement in detection sensitivity was obtained. Studies on the simultaneous speciation of elements by CE are scarce, however.

When analytes with or without UV absorbance co-exist in the same mixture, the indirect detection method will be problematic, or unnecessary. From this point of view, it should be reasonable to choose a reagent which can be used as detection-enhancing agent for the non-UV-active analytes as well as the running electrolyte additive to improve the separation. Sodium diethyldithiocarbamate has been used by Li et al. [6] as the off-column derivatization reagent for the analysis of organolead and tin compounds. EDTA has also been used for this purpose [11]. In that work, Cr(III), Fe(III), Cu(II) and Pb(II) were investigated using capillary zone electrophoresis (CZE).

Nitrilotriacetic acid (NTA) is a polyaminopolycarboxylic acid and is commonly used as an organic analytical reagent [12]. Independent experiments showed that it can form stable chelates with lead, mercury and selenium ions. Therefore, it was used in the present study for the simultaneous speciation of lead, mercury and selenium by CE. It was added to sample mixture as an off-column derivatization agent to form UV-absorbing chelates with the considered analytes. Moreover, it was also used as the running electrolyte additive to improve the resolution. A theoretical model was designed to demonstrate the influence of NTA, as well as pH and sodium dodecyl sulfate (SDS) on the migration behavior of the analytes. Field-amplified-injection was used for the on-line concentration of the analytes, to enhance the detection limits of the charged analytes. In this paper, we report the results of the foregoing experiments.

2. Theory

The electrophoretic mobility of inorganic cations, when complexing agent was used, has been studied in CZE [13]. In the case of both inorganic and organic species of multi-elements under study, a similar model is applicable for describing their migration behavior. When an electrolyte buffer is composed of a tri-carboxylic-group complexing agent (NTA), a surfactant agent, a buffer solution at a certain pH and the analytes, the following chemical equilibria are expected:

(i) Acid dissociation:

$$H_3L \rightleftharpoons H^+ + H_2L^- \quad K_1$$

$$H_2L^- \rightleftharpoons H^+ + HL^{2-} \quad K_2$$

$$HL^{2-} \rightleftharpoons H^+ + L^{3-} \quad K_3$$

where K_1, K_2 and K_3 are the corresponding dissociation constants, and their values in minus logarithm are 1.89, 2.49 and 9.73, respectively. In the pH range 3–9, HL^{2-} is the dominant form of NTA (Table 1). For simplicity, HL^{2-} is used for the following treatment.

(ii) Complexation of the analytes with NTA:

$$A^{b+} + HL^{2-} \rightleftharpoons AHL^{b-2}$$

Table 1
Log K values of NTA and the log β data of Pb(II), Hg(I) and Hg(II)

Constant	NTA	Pb(II)	Hg(II)	Hg(I)	PMA	Ref.
Log K_1, Log K_2, Log K_3	1.89, 2.49, 9.73	–	–	–	–	[12]
Log β_{AHL}	–	11.4	14.6	–		[12]
Log $\beta_{A(OH)m}$	–	6.2, 10.3, 13.3	10.3, 21.7	9	10	[15]

$$AHL^{b-2} + HL^{2-} \rightleftharpoons A(HL)_2^{b-4}$$

$$\beta_{AHL},\ \beta_{A(HL)2};\ b = 0, 1, 2$$

(iii) Inclusion complexation of the organometallic compounds with SDS:

$$A^{b+} + SDS^- \rightleftharpoons A^{b+}...SDS^- \quad \beta_{AS};\ b = 0, 1$$

(iv) Complexation of analytes with hydroxide:

$$A^{b+} + mOH^- \rightleftharpoons A(OH)_m^{b-m} \quad \beta_{AOH};\ m = 1, 2, 3$$

where A represents the analytes. β_{AHL}, $\beta_{A(HL)2}$ and β_{AS} are cumulative stability constants. 1:1 and 1:2 analyte–NTA complexes are taken into account in (ii) because complexes with higher ratios are difficult to obtain due to steric hindrance [12]. When the complexing agent is added in an equilibrium amount or in excess, the free analyte concentration will be so low that no polynuclear hydroxo complexes will be present [14]. Consequently, only mononuclear hydroxo complexes are considered here.

On the basis of the above equilibria, the concentrations of the various complexed species can be written as:

$$[AHL^{b-2}] = \beta_{AHL}[A^{b+}][HL^{2-}] \quad (1)$$

$$[A(HL)_2^{b-4}] = \beta_{A(HL)2}[A^{b+}][HL^{2-}]^2 \quad (2)$$

$$[A^{b+}...SDS^-] = \beta_{AS}[A^{b+}][SDS^-] \quad (3)$$

$$[A(OH)_m^{b-m}] = \beta_{AOH}[A^{b+}][OH^-]^m \quad (4)$$

Defining δ_L as the molar fraction of the complexing agent which is capable of reacting with the analytes, we have:

$$\delta_L = [HL^{2-}]/C_L, \text{ and } [HL^{2-}] = \delta_L C_L \quad (5)$$

where C_L represents the total concentration of the complexing agent.

The δ_L value of NTA is pH dependent. The higher the pH of the solution, the larger the δ_L, and the greater the complexing ability of NTA.

The total concentration of each analyte, C_A, is the sum of various chemical species present in the electrolyte buffer:

$$\begin{aligned} C_A &= [A^{b+}] + [AHL^{b-2}] + [A(HL)_2^{b-4}] + [A^{b+}...SDS^-] + [A(OH)_m^{b-m}] \\ &= [A^{b+}] + \beta_{AHL}\delta_L[A^{b+}]C_L + \beta_{A(HL)2}\delta_L^2[A^{b+}]C_L^{2+}\beta_{AS}[A^{b+}][SDS^-] \\ &\quad + \beta_{AOH}[A^{b+}][OH^-]^m \end{aligned} \quad (6)$$

Therefore, the molar fraction of the analytes in the free and various complexed species can be expressed by combining Eqs. (1)–(4), as:

$$\begin{aligned} \delta_A &= [A^{b+}]/C_A \\ &= 1/\left(1 + \beta_{AHL}\delta_L C_L + \beta_{A(HL)2}\delta_L^2 C_L^2 + \beta_{AS}[SDS^-]\right. \\ &\quad \left. + \beta_{AOH}[OH^-]^m\right) \end{aligned} \quad (7)$$

$$\begin{aligned} \delta_{AHL} = [AHL^{b-2}]/C_A = \beta_{AHL}\delta_L C_L/(1 + \beta_{AHL}\delta_L C_L + \beta_{A(HL)2}\delta_L^2 C_L^2 \\ + \beta_{AS}[SDS^-] + \beta_{AOH}[OH^-]^m) \end{aligned} \quad (8)$$

$$\begin{aligned} \delta_{A(HL)2} &= [A(HL)_2^{b-4}]/C_A \\ &= \beta_{A(HL)2}\delta_L^2 C_L^2/(1 + \beta_{AHL}\delta_L C_L \\ &\quad + \beta_{A(HL)2}\delta_L^2 C_L^2 \\ &\quad + \beta_{AS}[SDS^-] + \beta_{AOH}[OH^-]^m) \end{aligned} \quad (9)$$

$$\begin{aligned} \delta_{AS} &= [A^{b+}...SDS^-]/C_A \\ &= \beta_{AS}[SDS^-]/(1 + \beta_{AHL}\delta_L C_L + \beta_{A(HL)2}\delta_L^2 C_L^2 \\ &\quad + \beta_{AS}[SDS^-] + \beta_{AOH}[OH^-]^m) \end{aligned} \quad (10)$$

$$\delta_{AOH} = [A(OH)_m^{b-m}]/C_A$$
$$= \beta_{AOH}[OH^-]^m/(1 + \beta_{AHL}\delta_L C_L + \beta_{A(HL)2}\delta_L^2 C_L^2 + \beta_{AS}[SDS^-] + \beta_{AOH}[OH^-]^m) \quad (11)$$

The effective electrophoretic mobility, μ_{eff}, of each analyte is equal to the weighted average of the electrophoretic mobilities of the different free species of the analytes:

$$\mu_{eff} = \mu_0\delta_A + \mu_{AHL}\delta_{AHL} + \mu_{A(HL)2}\delta_{A(HL)2} + \mu_{AS}\delta_{AS} + \mu_{AOH}\delta_{AOH}$$
$$= \{\mu_0 + \mu_{AHL}\beta_{AHL}\delta_L C_L + \mu_{A(HL)2}\beta_{A(HL)2}\delta_L^2 C_L^2 + \mu_{AS}\beta_{AS}[SDS^-] + \mu_{AOH}\beta_{AOH}[OH^-]^m\}/\{1 + \beta_{AHL}\delta_L C_L + \beta_{A(HL)2}\delta_L^2 C_L^2 + \beta_{AS}[SDS^-] + \beta_{AOH}[OH^-]^m\} \quad (12)$$

where μ_0, μ_{AHL}, $\mu_{A(HL)2}$, μ_{AS} and μ_{AOH} are the electrophoretic mobilities of the various species. Eq. (12) gives a general expression of the migration behavior of analytes with consideration of the impact factors in the running electrolyte. Further, assuming only 1:1 complexes are formed between the analyte and complexing agent when the concentration of NTA in the electrolyte buffer does not exceed 20 times that of the total analytes, as was used in this work. Eq. (12) is simplified to:

$$\mu_{eff} = \frac{\mu_0 + \mu_{AHL}\beta_{AHL}\delta_L C_L + \mu_{AS}\beta_{AS}[SDS^-] + \mu_{AOH}\beta_{AOH}[OH^-]^m}{1 + \beta_{AHL}\delta_L C_L + \beta_{AS}[SDS^-] + \beta_{AOH}[OH^-]^m} \quad (13)$$

Eq. (13) indicates that the mobility of the analytes is related to the different β values, and the condition-dependent parameters, i.e., pH of the electrolyte buffer, concentrations of complexing agent and SDS. Changes in these factors will result in the variation in μ_{eff}.

3. Experimental

3.1. Instrumentation

A Prince (Emmen, Netherlands) CE system was employed in this work. The separation voltage was +20 kV. A Lambda 1000 (Bischoff, Leonberg, Germany) UV–Vis detector was used with the wavelength set at 200 nm for detection. Data acquisition was carried out with a Shimadzu C-R6A Chromatopac (Tokyo, Japan). The separation capillary was 63 cm (50 cm effective length)×50 μm I.D. Hydrodynamic injection of the samples was conducted by pressure (50 mbar for 0.1 min) at the anode end of the capillary. When amplified field sample injection (AFSI) was used for on-line sample stacking, a small plug of water was hydrodynamically injected (50 mbar, 0.1 min) just before the polarity-switching field-amplified injection of the solutes (+15 kV for 2.0 min and −15 kV for 1.97 min).

The capillary tube was conditioned daily by rinsing with solutions of 0.1 *M* sodium hydroxide (5.0 min), water (15 min) and finally the running electrolyte (30 min). Between two consecutive analyses, the capillary was flushed with water (3.0 min), 0.1 *M* sodium hydroxide (3 min), water (4 min) and the running electrolyte (15 min) to improve the reproducibility of the EOF and migration time of the analytes.

3.2. Chemicals and materials

Trimethyllead chloride (TMLC), triethyllead chloride (TELC), diphenyllead dichloride (DPLC), phenylselenium chloride (PSC), diphenylselenium (DPS), phenylmercuric acetate (PMA) and selenium powder were purchased from Aldrich (Milwaukee, WI, USA). Lead(II) nitrate, mercury(II) chloride and mercury(I) nitrate were supplied by J.T. Baker (Phillipsburg, NJ, USA). SDS was obtained from Fluka (Buchs, Switzerland), and NTA was purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade.

Stock solutions of the organometallic compounds were prepared in methanol at concentrations of 5080 μg/ml for TMLC, 5900 μg/ml for TELC, 2550 μg/ml for DPLC, 2650 μg/ml for PSC, 650 μg/ml for PMA and 550 μg/ml for DPS. The stock solutions of Hg(I), Hg(II) and Pb(II) were prepared by dissolving the corresponding salts in deionized water, and had concentrations of 1040 μg/ml, 600 μg/ml and 560 μg/ml, respectively; 1000 μg/ml of selenium(IV) stock solution was obtained by dissolving selenium powder in concentrated nitric acid under heating, followed by diluting with water; 50 mmol/l (m*M*) NTA aqueous solution was also prepared. In order to increase its solubility in the

water, 1.0 *M* NaOH was added to the solution during the preparation.

Off-column derivatization of samples was carried out by mixing the appropriate volumes of the stock solutions with 5.0 m*M* NTA in a 40 m*M* pH 6.0 NaH_2PO_4–Na_2B4O_7 buffer solution; 40 m*M* NaH_2PO_4–$Na_2B_4O_7$ solution was used in the running electrolyte at different pH values. It was prepared by mixing 100 m*M* sodium tetraborate solution with 500 m*M* phosphoric acid according to the pH required, and diluted before use. Deionized water used throughout the experiment was produced on a Milli-Q system (Millipore, Bedford, MA, USA).

3.3. Calculation

In practice, the electrophoretic mobility of each analyte, μ_{eff}, is calculated from:

$$\mu_{eff} = \frac{L_t L_d}{V}\left(\frac{1}{t} - \frac{1}{t_0}\right)$$

where L_t and L_d are the total and effective length of the capillary, respectively. V is the applied voltage (20 kV), t the migration time of the analyte and t_0 the migration time of methanol, which is used as the (EOF) marker. The corrected peak area was used to evaluate the effect of different parameters on the detection of the analytes, and detection enhancement by stacking injection [9].

4. Results and discussion

4.1. Effect of pH

Under CZE, the fourth term with reference to the effect of SDS on μ_{eff} in Eq. (13) can be omitted. At low pH (<4), μ_0 and the terms referring to the influence of hydroxide can be neglected, provided that the β_{AHL} of the analyte–NTA complexes is large enough (e.g., $>10^{10}$ in Table 1) and NTA concentration not so low (e.g., in m*M* range). Under these conditions, Eq. (13) can be simplified to $\mu_{eff} = \mu_{AHL}$. This means that the analytes were fully complexed with NTA. On the other hand, μ_{eff} can be approximated as μ_{AOH} under very basic conditions (pH$>$9). Practically, neither pH value is applicable because of the poor detection sensitivity and slow analytical speed or precipitation of the analytes. It seemed that pH values in the medium range are appropriate. The effect of the running electrolyte pH upon the electrophoretic mobility and resolution was first conducted under weak acid to basic conditions, ca. pH 5.0 to 8.0, and the mobility–pH curves were plotted in Fig. 1. The nearly zero-mobility of diphenylselenium (DPS) denoted its electrical neutrality and migration at EOF. The positive signs in the mobility of alkyllead (TMLC and TELC) indicate their electrical positivity and faster migration than the others. Their mobilities decreased with the increase of pH. It has been reported that both TMLC and TELC would ionize and form ion-pairs with the buffer anions in the aqueous solution [16]. The greater decrease in mobility of TMLC than that of TELC could be attributed to weaker steric hindrance caused by the trimethyl group than that of the triethyl group, and its more favourable association with anions. The decrease in mobility above a certain pH value for DPLC (5.75), PSC (6.0) and PMA (6.0) showed an increased influence of hydroxide, as in Eq. (13). For the inorganic ions, Hg(II), Pb(II) and Hg(I) had decreased mobility with the increase of

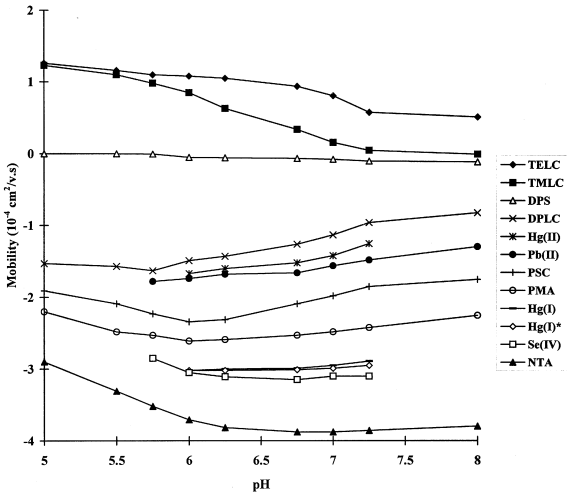

Fig. 1. Effect of pH on mobility. Running electrolyte: 40 m*M* pH 5.0–8.0 NaH_2PO_4–$Na_2B_4O_7$ buffer+1.0 m*M* NTA; column: fused-silica capillary (63 cm×50 μm I.D.) with 50 cm length for separation; injection: 50 mbar×0.1 min; voltage: 20 kV; detection: 200 nm. Sample mixture: 40 m*M* pH 5.5 NaH_2PO_4–$Na_2B_4O_7$ buffer+1.0 m*M* NTA+analytes.

pH, due to their increased cumulative stability constants of hydroxo-complexes under the working conditions. Se(IV) was present as SeO_3^{2-}. It has a similar mobility–pH curve as NTA. It was also noticed that the formation of metal ion–NTA complexes was pH dependent. Compared to the organometallic species, inorganic ions were detectable in a narrower pH range, e.g., Hg(I) and Hg(II) (pH 6.0 to 7.25), Se(IV) (5.75 to 7.25) and Pb(II) (5.75 to 8.0). Double peaks were observed for Hg(I). A possible explanation for this observation is that it forms multi-ligand complexes with NTA. This can not be confirmed as yet.

Overall, the influence of pH on the mobility is complicated. Changing the pH will produce different values of μ_{AHL}, μ_{AOH}, β_{AHL}, β_{AOH}, δ_L, $[H_3L]$ and $[OH^-]$ as in Eq. (13), and hence, different trends of the variation in mobility, as has been verified by the multiple mobility–pH curves in Fig. 1.

Under pH 7, the migration of the analytes increased according to the following order: NTA (the slowest), Se(IV), Hg(I), PMA, PSC, Pb(II), Hg(II), DPLC, DPS, MeOH, TMLC and TELC (the fastest).

Because the formation of complexes is pH dependent, it is reasonable that the change of the running electrolyte pH will also affect the detection of the analytes. Indeed, the corrected peak areas of metal ion–NTA complexes increased with the increase of pH until 6.5, and then decreased slightly. Peak areas of TELC, DPLC and DPS were not sensitive to the variation of pH. However, it decreased for TMLC when the pH of the running electrolyte was higher than 7. A possible explanation is that the ion-pair formed between TMLC and borate ion leads to a reduction in its absorbance. Enhanced detection sensitivities were observed for PSC and PMA with the increase of pH. In our opinion, this is due to the formation of their NTA complexes.

The pH range of 6–7.5 was acceptable for the separation and detection of organic compounds. For the purpose of simultaneous speciation of all the analytes, a narrower pH range should be employed (6.5–7.25). In the present work, pH 7 was chosen.

4.2. Effect of SDS

Under CZE conditions, separation among TMLC, MeOH and DPS, as well as Hg(II) and Pb(II) could not be achieved. Besides, TELC migrated prior to MeOH, and peak tailing was also noticed for Hg(I). To improve the separation, MEKC was performed. Accordingly, if SDS concentration is the only variable, Eq. (13) can be rewritten as:

$$\mu_{eff} = \frac{\mu_0 + \mu_{AHL}\beta_{AHL}\delta_L C_L + \mu_{AS}\beta_{AS}[SDS^-] + \mu_{AOH}\beta_{AOH}[OH^-]^m}{1 + \beta_{AHL}\delta_L C_L + \beta_{AS}[SDS^-] + \beta_{AOH}[OH^-]^m} = \frac{\mu_0^* + \mu_{AS}\beta_{AS}[SDS^-]}{B + \beta_{AS}[SDS^-]} \quad (14)$$

where

$$\mu_0^* = \mu_0 + \mu_{AHL}\beta_{AHL}\delta_L C_L + \mu_{AOH}\beta_{AOH}[OH^-]^m,$$

and

$$B = 1 + \beta_{AHL}\delta_L C_L + \beta_{AOH}[OH^-]^m.$$

For the inorganic metal ions which do not react with SDS, e.g., Hg(II), Pb(II), Hg(I), Se(IV), or those organometallic compounds which SDS exerts negligible influence on their mobilities, Eq. (14) can be further approximated as $\mu_{eff} = \mu_0^*/B$. It means that their mobilities are not affected by the variation of SDS concentration; for the organometallic compounds whose migrations are greatly affected by SDS, distinct changes in their mobilities will be predictable before the formation of stable SDS–inclusion complexes, e.g., SDS lower than its critical micelle concentration (CMC). In the cases that the concentration of SDS exceeds the equilibrium concentrations of forming stable inclusion complexes, the analyte–SDS inclusion complexes will be dominant. Consequently, $\mu_0^* \ll \mu_{AS}\beta_{AS}[SDS^-]$ and $B \ll \beta_{AS}[SDS^-]$; a simplified Eq. (14) is achieved as $\mu_{eff} = \mu_{AS}$. This means their mobilities are independent of SDS concentration.

The effect of SDS concentration on the mobility of the analytes is plotted in Fig. 2. Agreement with the above prediction was obtained for the inorganic species, DPLC, PSC and PMA. The observation showed that SDS exerted very slight influence on them. TMLC, TELC and DPS have different mobility–SDS concentration curves. Their mobilities were very sensitive to SDS concentration, and seemed to suggest that TMLC, TELC and DPS formed inclusion complexes with SDS in preference to complexing with NTA. Also, differences existed in their mobility variation. From TMLC, TELC to DPS, the

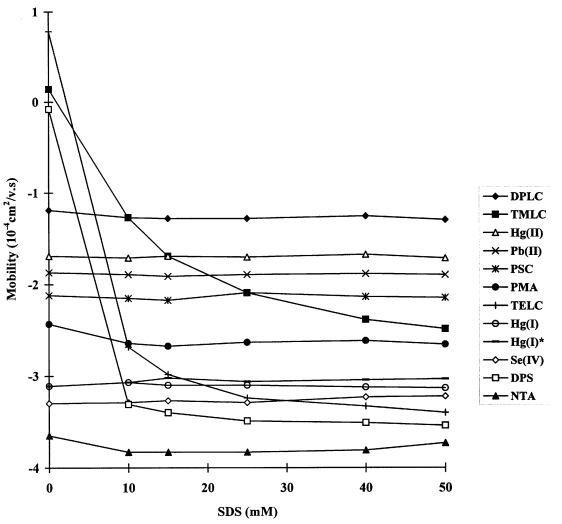

Fig. 2. Effect of SDS on mobility. Running electrolyte: 40 m*M* pH 7.0 NaH_2PO_4–$Na_2B_4O_7$ buffer+1.0 m*M* NTA+0–50 m*M* SDS. Other conditions as in Fig. 1, except pH 7.0 in sample mixture.

required SDS concentration to make them to reach their stable mobilities decreased. This is indicated by the observation that the mobility of DPS was nearly constant after 10 m*M* SDS, and that of TELC varied until 25 m*M* SDS, whereas the mobility of TMLC increased continuously under the working conditions. The negligible variation of the mobility of the other analytes showed that SDS concentration exerted very little influence in these cases.

In CE, the use of SDS as a surfactant to perform MEKC has been widely studied. However, its influence on detectability was seldom considered. This should be reasonable when (i) the analytes under investigation, as in most cases, have strong UV absorbing groups, and their detection is not a problem; (ii) the detection wavelength >220 nm was chosen for determination. Thus, the UV absorbance effect of SDS can be ignored. Actually, many CE analyses have usually been different from the above. For example, it was not uncommon to employ a shorter wavelength (200–220 nm) for sensitive detection [17,18]. Therefore, the effect of SDS on the detection sensitivities should be taken into consideration, especially for the analytes which are UV transparent, such as inorganic ions, TELC and TMLC considered in this work.

Generally, there are two aspects of the influence of SDS on detection: (i) the increased background absorbance and deterioration of detection sensitivity (this has been verified through the rising background absorbance of the conditioned capillary tubing before electrophoresis). The higher the SDS concentration, the higher the background absorbance will be; (ii) the enhancement in the detection of analytes by SDS associating or forming inclusion complexes. Maximum enhancement of detection was obtained for the analytes at a certain SDS concentration. Results showed that the detection was enhanced for most analytes after the addition of SDS in the running electrolyte. Their peak areas reached the maximum values at specific SDS concentrations, and decreased at higher SDS concentrations. After the corrections of the peak areas, the obtained improvement of detection for the analytes was different, according to the variation of SDS concentration, ranging from a slight improvement [Hg(II), Pb(II) and Hg(I)], to ~1.5-fold (PSC and PMA), ~2-fold (DPLC, TMLC and DPS), to nearly 7-fold improvement (TELC).

4.3. Effect of NTA

The effect of NTA upon the determination was firstly evaluated by comparing the separation and determination of the solutes with no NTA being used, NTA used under off-column derivatization conditions, or NTA used under on-column derivatization conditions (Fig. 3). There was no peak belonging to Pb(II), Hg(II), Hg(I), DPLC and PMA in the electropherogram (Fig. 3a) when no NTA was used. Under off-column derivatization (Fig. 3b), incomplete complexation between DPLC and NTA was expected from its low peak height. Besides, the co-migrating Hg(II)–NTA and Pb(II)–NTA revealed that their electrophoretic mobilities were too similar to be resolved. Although only on-column derivatization permitted the determination of all analytes (Fig. 3c), the small peaks of Hg(II), Pb(II), Hg(I) and DPLC indicated their incomplete complexation with NTA. For the simultaneous speciation of all the analytes, it seems necessary to use NTA both as the off-column derivatization agent and the running electrolyte additive.

Under conditions when NTA is the only variable, Eq. (13) can be simplified to:

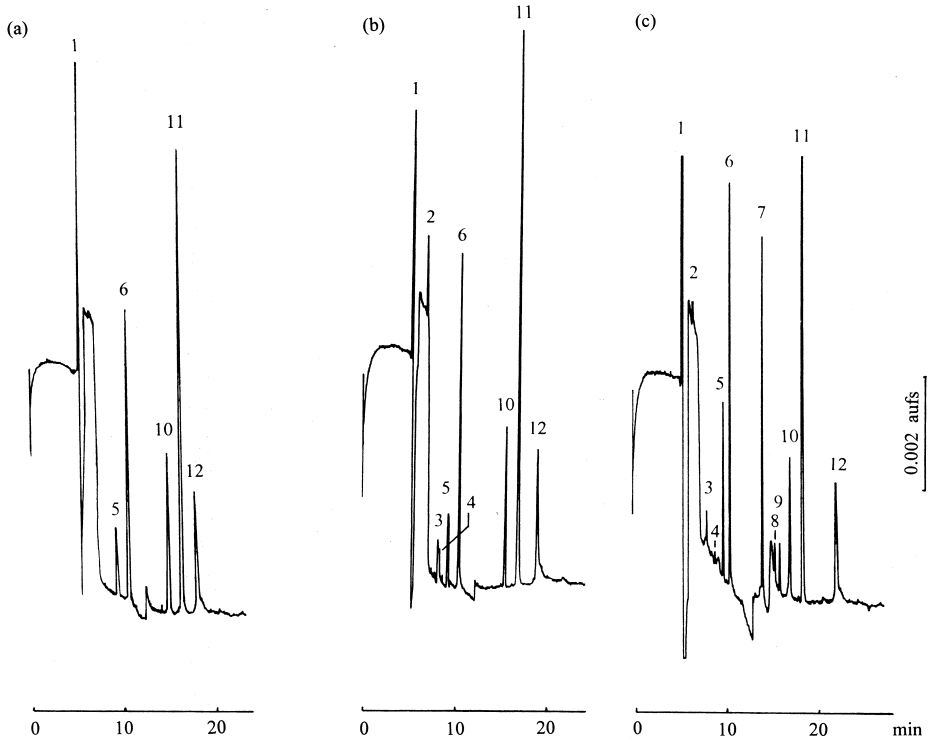

Fig. 3. Difference for resolution and detection among derivatization methods. Running electrolyte: 40 m*M* pH 7.0 NaH_2PO_4–$Na_2B_4O_7$ buffer+40 m*M* SDS+NTA (a and b: 0 m*M*; c: 7.5 m*M*). Sample mixture: 40 m*M* pH 6.0 NaH_2PO_4–$Na_2B_4O_7$ buffer+NTA (a and c: 0 m*M*; b: 7.5 m*M*). Peaks (concentration): 1=MeOH; 2=DPLC (63.75 μg/ml); 3=Hg(II) (22.1 μg/ml); 4=Pb(II) (17.9 μg/ml); 5=PSC (66.25 μg/ml); 6=TMLC (63.5 μg/ml); 7=PMA (65 μg/ml); 8 and 9=Hg(I) (39.7 μg/ml); 10=Se (IV) (15 μg/ml); 11=TELC (68 μg/ml); 12=DPS (55 μg/ml); 13=NTA. Other conditions as in Fig. 1.

$$\mu_{\text{eff}} = \frac{\mu^{**} + \mu_{\text{AOH}}\beta_{\text{AOH}}[\text{OH}^-]^m + \mu_{\text{AHL}}\beta_{\text{AHL}}\delta_{\text{L}}C_{\text{L}}}{B^* + \beta_{\text{AOH}}[\text{OH}^-]^m + \beta_{\text{AHL}}\delta_{\text{L}}C_{\text{L}}} \quad (15)$$

where $\mu^{**}=\mu_0+\mu_{\text{AS}}\beta_{\text{AS}}[\text{SDS}^-]$ and $B^*=1+\beta_{\text{AS}}[\text{SDS}^-]$.

Eq. (15) shows that μ_{AHL} will be independent of the concentration of NTA if stable complexes were formed. Consequently, the influence of NTA on μ_{eff} will become insignificant with further increase in ligand concentration.

Agreement with the above prediction was observed, when NTA concentration in the running electrolyte was investigated, ca. from 0 to 7.5 m*M* (Fig. 4), as a factor on the mobility of analytes. Different trends in the variation of mobility was obtained at lower NTA concentrations, e.g., <1.0 m*M*. However, all the curves, except PMA, were nearly horizontal when NTA concentration was higher than 1.0 m*M*. This indicated that stable NTA complexes of the analytes were formed under those conditions. For PMA, it is believed that stable NTA complex did not form until ~2.5 m*M* NTA was used.

Experimental results also revealed that the increase in NTA concentration is helpful for the improvement of detection sensitivity of the analytes. In this work, the largest peak areas were obtained for most of the analytes when 5 m*M* NTA was used. Further increase of complexing agent in the running electrolyte led to a deterioration of the detection of some analytes and prolonged the total elution time.

The impact of NTA was also evaluated on the migration and detection of the analytes at different concentrations in the sample mixture (0, 1, 2.5, 5.0 and 7.5 m*M* NTA). Results showed that its influence

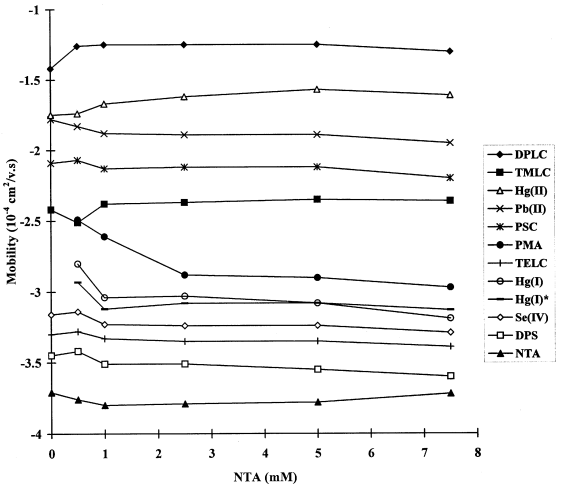

Fig. 4. Effect of NTA in the running electrolyte. Running electrolyte: 40 m*M* pH 7.0 NaH_2PO_4–$Na_2B_4O_7$ buffer+40 m*M* SDS+0–7.5 m*M* NTA. Other conditions as in Fig. 3 except NTA concentration 2.5 m*M* in the sample mixture.

was similar to when it was used in the running electrolyte. In order to match the optimal NTA concentration used in the running electrolyte, 5 m*M* NTA should be used.

A typical electropherogram of the simultaneous speciation of ten analytes and NTA is given in Fig. 5. Baseline separation was achieved for all components within 25 min. Under the optimal conditions, a dominant complex was formed for Hg(I) indicated by the main peak in the electropherogram. Approaches such as employing capillaries of shorter length, higher operating voltage or even both are possible for further improving on the total running time.

4.4. Enhancement of detection limits of analytes with AFSI

AFSI has been reported to be an effective injection mode for the on-line stacking of electrically charged solutes [19,20]. When NTA was used as the off-column derivatization agent, electrically charged complexes were formed between the ligand and the analytes, making it feasible for using AFSI for the on-line stacking of these complexes and enhancing their detection. A typical electropherogram of the solutes obtained under AFSI is shown in Fig. 6.

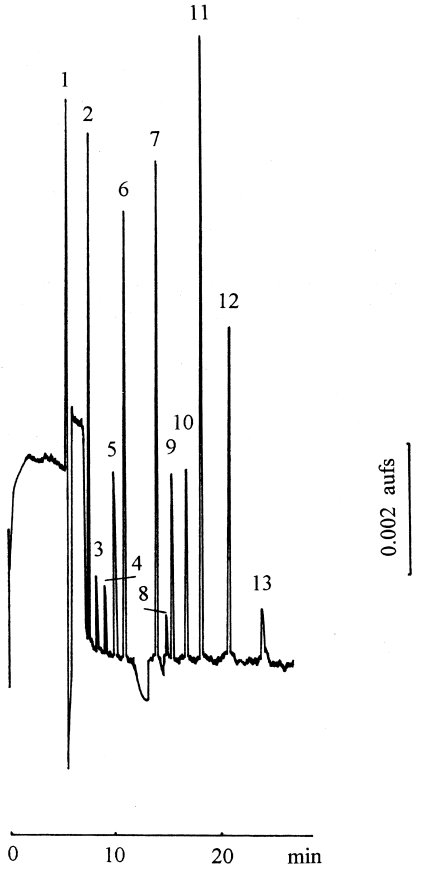

Fig. 5. Electropherogram of ten species using hydrodynamic injection. Running electrolyte: 40 m*M* pH 7.0 NaH_2PO_4–$Na_2B_4O_7$ buffer+40 m*M* SDS+5.0 m*M* NTA. Sample mixture: 40 m*M* pH 6.0 NaH_2PO_4–$Na_2B_4O_7$ buffer+5.0 m*M* NTA. Other conditions as in Fig. 3 except: DPS 48.0 μg/ml; PMA 30.0 μg/ml; PSC: 47.5 μg/ml and TELC 30.0 μg/ml.

Enrichment was achieved for DPLC, PSC, PMA, Hg(I) and Se(IV). However, broad peaks were observed for the other solutes. Theoretically, AFSI is based on the increased electrophoretic velocity, which is induced by the enhanced electric field in a diluted sample matrix, of electrically charged solutes [21]. Besides the enrichment of analytes, the stacking process also causes peak narrowing. However, the laminar flow, which is generated from the mismatch between the local EOF in each individual region and the bulk velocity of the fluid inside the column, will broaden the sharp zone yielded during the stacking process. Therefore, the actual enrichment efficiency and peak shape of AFSI are controlled by these two

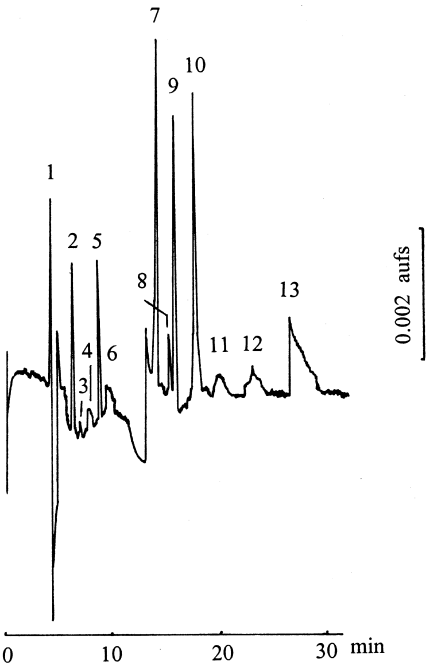

Fig. 6. Electropherogram generated using AFSI. 50 mbar×0.15 min for water plug; +20 kV 2.0 min and −20 kV 1.90 min for sample stacking. Concentrations of the sample matrix and all solutes were 1/40 and 1/200, respectively, as for Fig. 5. Other conditions as in Fig. 5.

opposite effects. In AFSI, conditions which usually influence stacking include the field enhancement factor (represented by the ratio of the buffer concentration in sample solution to that in the column), stacking voltage and stacking time [20]. In addition, the characteristics of the analytes also play a role on their enrichment. It has been reported that the injection of analytes having a higher mobility will be favored [22]. From the above discussion, two reasonable extrapolations could be made that: (i) AFSI is less effective for neutral or very low charge-to-mass ratio compounds; (ii) the laminar flow will cause much more peak broadening for neutral or very low charge-to-mass ratio compounds because of their less effective charges. As has been shown previously, DPS was present as neutral form in sample solution; for TMLC and TELC, their association with borate ion or NTA reduced their effective charges, and the hindrance of trialkyl groups reduced their mobility. Subsequently, less stacking efficiency as well as broadening peaks was obtained. For Hg(II) and Pb(II), this phenomenon should be due to their forming electrically neutral complexes with NTA (present as the 2^- anion under the experimental conditions).

The effect of AFSI was compared with hydrodynamic injection, and corresponding results are listed in Table 2. A 40–600 fold enhancement in detection can be obtained for different analytes.

The AFSI in the method also provided a viable approach for the determination of the complexation rate. For example, the obtained electropherogram shows that Hg(II) and Pb(II) formed 1:1 complexes with NTA. Furthermore, it could be possible to determine whether the complexes were positively or negatively charged by choosing the appropriate AFSI modes.

5. Conclusions

We have reported an effective CE method for the simultaneous speciation of elements, using NTA as an off-column derivatization reagent and as a running electrolyte additive. UV absorbing complexes

Table 2
Limits of detection (LODs) for ten species

	LOD (ng/ml)									
	Compounds									
	Pb(II)	TMLC	TELC	DPLC	Hg(II)	Hg(I)[b]	PMA	Se(IV)	PSC	DPS
A[a]	110	80	40	100	130	310	90	120	260	10
AFSI	–	–	–	2.48	–	0.54	0.41	0.2	5.31	–
Enrichment (fold)	–	–	–	44	–	562	225	608	49	–

[a] A: Hydrodynamic injection (50 mbar×0.1 min).
[b] Main peak was used for calculation.

were formed between the ligand and the solutes, and direct determination could be performed. AFSI permitted on-line stacking of the electrically charged species–NTA complexes. Detection limits at the ng/ml level were obtained. On the basis of multi-chemical equilibria in the electrolyte buffer, a theoretical model was established to describe the migration behavior, μ_{eff}, of the analytes. By applying the model, parameters which affect the derivatization, separation and determination were optimized, and simultaneous speciation was achieved. Besides being used to perform MEKC for improving separation, SDS also enhanced the detection of the analytes. Moreover, this method provided useful information about the complexing rates of some cations. Further work will be focused on the applications of this method.

Acknowledgements

The authors thank the National University of Singapore for financial support.

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