CAPILLARY ELECTROPHORESIS AS A TOOL FOR SPECIATION ANALYSIS OF HEAVY METALS

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SUMMARY

Separations of chloroaquachromium(III) ions, cobaltammines and triorganotin compounds are presented as examples of the application of capillary zone electrophoresis with UV detection in speciation studies. Separation of tributyl- and triphenyltin was obtained with tartaric acid electrolyte; camphorsulphonic acid was a satisfactory running electrolyte for all the other separations.

INTRODUCTION

For many years it has been widely recognized that proper evaluation of the role of a given element in the natural environment or in living organisms requires its speciation besides knowledge of its total content. Speciation may be defined as the process of identifying and quantifying different, defined species, forms or phases present in a given material [1]. Among the various forms in which heavy metals occur in the environment, especially common is their presence in complex compounds of different stability formed with numerous ligands. Chromatographic methods are of particular importance in speciation studies because they often enable simultaneous determination of a variety of chemical species containing the same element in their molecule [2,3]. Among the chromatographic techniques column methods predominate; these are most commonly hyphenated with atomic or mass spectrometry, which increase the ‘speciation power’ of the complete measurement system. The most significant advantage of the use of high-performance column chromatography for speciation is usually the very limited disturbance of ionic and redox equilibria existing in natural media.

High-performance capillary electrophoresis (CE), in which electrophoretic migration is influenced by the electric charge and radius of
the separated ions, seems to offer even broader possibilities for speciation. Intensively developed in recent years, primarily for the analysis of biological macromolecules, it is nowadays employed in separation of various kinds of chemical compound, including inorganic ions and also even whole cells and virus particles [4,5]. The mechanisms responsible for separation in capillary electrophoresis are different from those in liquid chromatography. Separation is based on differences in analyte velocity in an electric field; this is also modified by electroosmotic flow resulting from the effect of the applied electric field on the solution double-layer at the capillary wall. In comparison with HPLC, capillary electrophoresis can be considered as a complementary methodology, with usually simpler method development, smaller sample-volume requirements and almost always better resolving power. Analysis times are usually shorter and hyphenation to mass spectrometry is simpler.

Numerous papers have already been published on the use of CE for analysis of metal cations; these have usually been based on separations employing capillary zone electrophoresis (CZE) with the use of homogeneous buffer systems. Selectivity in such a separation can be modified by changing the buffer pH, addition of surfactants or complexing ligands. The use of a running electrolyte of appropriate composition enables simultaneous determination a large number of cations including those of alkali metals, alkaline earth metals, transition metals and lanthanides [e.g. 6-8]. All these separations were developed with indirect UV detection. Recently very satisfactory prediction and optimization of the separation of metal cations by CE has also been reported [9].

Several papers have recently been devoted to capillary electrophoresis of metal complexes. CZE has been used for the separation of transition metal complexes with 2,2′-dihydroxyazobenzene-5,5′-disulphonate [10] and for the separation of cyanide complexes [11]. The use of surfactants in the running buffer in micellar electrokinetic chromatography enables the separation both of charged and neutral species. This technique, which can be considered as a hybrid of electrophoresis and chromatography, has been used for the separation of negatively charged chelates with PAR [12,13], of arsenazo(III) complexes [13] and of α,β,γ,δ-tetrakis(4-carboxyphenyl) porphine [14].

Several papers have been published dealing with the use of CE in the separation of metallic elements, several different chemical species of the same element being determined in a single run. CZE has been employed for the speciation of iron cyanide complexes [15], for the speciation of
anionic forms of arsenic [16,17] and selenium [17], for the separation of diastereomeric platinum [17] and cobalt(III) [18] complexes and for the speciation of aluminium [19,20] and cysteine complexes of organic mercury compounds [21]. Micellar electrokinetic chromatography has been used for the determination of organolead and organoselenium compounds [22], organic and inorganic arsenious and selenious compounds [23] and also for ferrocene derivatives [17].

The aim of this study was to investigate the possibility of using capillary electrophoresis for several other speciation problems not yet reported in the literature.

EXPERIMENTAL

Apparatus

The electrophoresis system used in this study was an ISCO (Lincoln, NE, USA) model 3850 with output connected to a Linear strip chart recorder. Separations were performed in 70-cm capillaries of 75 µm i.d.; the length to the detector was 60 cm. Sample introduction, with 1- and 10-mL syringes from Unimetrics, was performed with a split ratio of 379:1.

Preconcentration of triorganotin compounds was performed on laboratory-made microcolumns packed with Amberlite XAD-2 resin.

Reagents

(1S)-(+)-Camphorsulphonic acid (CSA) was purchased from Sigma, tartaric acid and butyltrimethylammonium chloride (BTAC) from Aldrich, and β-cyclodextrin from Kodak. The non-polar resin Amberlite XAD-2 was from Fluka.

CrCl₃·6H₂O was from Germed (Dresden, Germany). Published procedures were used for the synthesis of [Co(NH₃)₆]Cl₃, [Co(NH₃)₃Cl]Cl₂, [Co(NH₃)₄CO₃]NO₃ and trans-[Co(en)₂Cl₂]Cl [24], [Co(NH₃)₄(H₂O)₂]₂(SO₄)₃ [25], and [Co(NH₃)₅C₂O₄H](NO₃)₂ [26].

Organotin compounds were the kind gift of Dr Norbert Buschmann of the Department of Analytical Chemistry, University of Münster, Germany.

All other reagents used were of analytical grade, purchased from POCh (Gliwice, Poland). All solutions were prepared from deionized water from a Millipore Milli-Q system. Eluents were filtered through 0.45 µm nylon filters (Millipore) and degassed in an ultrasonic bath before use.
Procedures

Before analysis, capillaries were cleaned by rinsing for 30 min with each of 1 M NaOH and 1 M HCl and overnight with water. Before sample introduction the column was stabilized with the electrolyte used for at least 30 min.

Preconcentration of organotin compounds was performed with a microcolumn containing dry Amberlite XAD-2 (0.5 g). Samples of natural waters (1 L) spiked with trimethyl-, triethyl- and tributyltin chlorides (2 µg L⁻¹) and triphenyltin chloride (0.2 µg L⁻¹) were preconcentrated at a flow-rate of 7 mL min⁻¹. Preconcentrated species were eluted with methanol (10 mL), evaporated to approximately 1 mL and diluted to 2 mL with methanol before injection.

RESULTS AND DISCUSSION

Separation of chloroaquachromium(III) complexes

Chromium(III) forms complexes with any species capable of donating an electron pair. Some exhibit hydrate isomerism, which occurs when water may be inside or outside the coordination sphere. One example is CrCl₃·6H₂O, which exists in one of three forms [Cr(H₂O)₆]Cl₃, [CrCl(H₂O)₅]Cl₂·H₂O and [CrCl₂(H₂O)₄]Cl·2H₂O. The compound usually designated as CrCl₃·6H₂O in the solid state contains discrete trans-[CrCl₂(H₂O)₄]⁺, Cl⁻ and H₂O units [27]. In aqueous solution dichlorotetraaquachromium(III) reacts to form [CrCl(H₂O)₅]⁺⁺ with a half-life of approximately 2.5 h. Further reaction to hexaaquachromium(III) proceeds with a half-life of about 700 h. Particular chloroaquachromium(III) ions can be isolated by ion-exchange fractionation.

The presence of these various species in solution can be investigated with capillary electrophoresis which gives good resolution because of the different electric charges on the ions. A 1 mM solution of camphorsulphonic acid of pH 3.0 was used as running electrolyte because this had previously been used successfully as eluent for high-performance ion-exclusion chromatography of carboxylic acids and inorganic anions [28]. Fig. 1 shows the electropherogram obtained from a 1 mM aqueous solution of CrCl₃·6H₂O 1 h after dissolution of the solid reagent. Peaks 1 and 2 correspond to the monochloro- and dichlorochromium(III) ions, respectively. The large signal which appears as peak 3 corresponds to the
electroosmotic flow boundary. No signal corresponding to the hexaaquachromium(III) ion was observed under these conditions; this is in agreement with the known chemistry of this system.

**Separation of cobalt(III) complexes**

Ammino complexes of cobalt(III) are known examples of ionization isomerism, in which the complex ion is in solution with a counter ion, which is itself a potential ligand. Because of the inertness of the cobaltammines, capillary electrophoresis can again be used to demonstrate the presence in solution of several forms, which differ in number of ligands and in electric charge.

CZE separation of a 1 mM mixture of complexes with different numbers of chloride ligands, which were prepared separately, was also performed using camphorsulphonic acid solution as electrolyte. Baseline resolution was obtained for 1 mM CSA at 30 kV (Fig. 2). As is demonstrated in Fig. 3A, an increase in electrolyte concentration results in longer migration times for all the separated species. This could be a consequence of two different phenomena: a decrease in the thickness of the diffusion layer leading to a decrease in the electrokinetic potential or a decrease in pH resulting in greater dissociation of functional groups. This results in lower rate of electroosmotic flow and longer migration times, which consist of electroosmotic and electrophoretic components. Increasing migration times were also observed when the applied voltage was reduced.

CZE under the same conditions can be used for satisfactory separation of a much more complicated mixture of Co(III) complexes (Fig. 4). The complexes are separated in accordance with the different electric charges of the complexing cations and as a result of the presence of different ligands in the coordination sphere. The species analysed are separated into three groups according to electric charge (+3, +2 and +1) and an additional peak is observed for the sample solvent. According to the expression for electrophoretic mobility:

\[ \mu_{ef} = \frac{z_i e}{6\pi \eta R} \]

where \( z_i \) is the ionic charge, \( e \) the charge on the electron, \( \eta \) the viscosity of the running electrolyte and \( R \) the ionic radius; changing the electric charge from +1 to +2 doubles the electrophoretic mobility. Much smaller differences in migration times are observed for complexes with
different ligands, because differences between their sizes are much smaller.

Separation of triorganotin compounds

Differences in the migration times of trialkyltin chlorides result from the presence of different alkyl groups in organotin cations of the same electric charge. CZE separation of trialkyl derivatives can also be performed satisfactorily with the use of 1 mM solution of camphorsulphonic acid as running electrolyte. Because of the optical properties of trialkyltins it is necessary to use indirect UV detection for this analysis; 1 mM butyltrimethylammonium chloride (BTAC), which has an absorption maximum at 218 nm, is added to the electrolyte. The electropherogram obtained (Fig. 5A) shows satisfactory resolution of all three trialkyltins. The addition of 10 mM β-cyclodextrin to the electrolyte, as previously employed for CZE of organolead and organoselenium compounds [18], increases migration times slightly because of the increase in the viscosity of the solutions, but does not significantly influence the quality of the separation of the trialkyltins (Fig. 5B). Most probably the size of organotin analytes is too large in comparison with that of the internal torus of the β-CD.

For environmental purposes it is essential to be able to determine tributyltin (TBT) and triphenyltin (TPT) at ppb or sub-ppb levels. Under the experimental conditions described above these two organotin compounds cannot be separated. Satisfactory resolution, although with much longer migration times, can be achieved by using as running electrolyte a 20 mM solution of tartaric acid in 4:1 water-methanol containing 4 mM BTAC. Under these conditions all triorganotin compounds examined can be well resolved (Fig. 6); in order to obtain sufficient detectability, however, preliminary preconcentration is needed. This can be achieved by solid-phase extraction on microcolumns packed with non-polar Amberlite XAD-2; 500-fold preconcentration is obtained by use of a 500 mg resin bed. Experiments performed with natural water samples spiked with 2 μg L⁻¹ TBT and 0.2 μg L⁻¹ TPT indicate that the efficiency of preconcentration is satisfactory; the method could, therefore, find practical application in environmental analysis.
CONCLUSIONS

The examples shown above and those, mentioned in the introductory section, cited from the publications of other authors clearly indicate that high-performance electrophoresis can be considered as a new powerful tool for speciation studies. It can be also expected that this area of its application will rapidly grow in the near future with progress in detection methods which can be adapted to this separation technique.

ACKNOWLEDGEMENT

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Fig. 1
Electropherogram obtained from a 1 mM solution of CrCl$_3$·6H$_2$O in water using 1 mM CSA as running electrolyte. Voltage applied, 20 kV. Detection at 210 nm. Peaks: 1, [CrCl(H$_2$O)$_5$]$^{2+}$; 2, [CrCl$_2$(H$_2$O)$_4$]$^+$; 3, electroosmotic flow boundary.
Fig. 2
Electropherogram obtained from a mixture of 1 mM cobaltammines using 1 mM CSA as running electrolyte. Voltage applied, 30 kV. Detection at 200 nm. Peaks: 1, [Co(NH$_3$)$_6$]$^{3+}$; 2, [Co(NH$_3$)$_5$Cl]$^{2+}$; 3, [Co(NH$_3$)$_4$Cl$_2$]$^+$; 4, electroosmotic flow boundary.
Fig. 3

Effect of CSA concentration in the running electrolyte (A) and of applied voltage (B) on the migration times of cobaltammines: [Co(NH$_3$)$_6$]$^{3+}$ ( ○ ), [Co(NH$_3$)$_5$Cl]$^{2+}$ ( □ ) and [Co(NH$_3$)$_4$Cl$_2$]$^{+}$ ( Δ ). Measurements were performed at 20 kV (A) and using 1 mM CSA (B). Detection at 210 nm.
Fig. 4
Electropherogram obtained from a mixture of 1 mM cobaltammines: 1, [Co(NH$_3$)$_6$]Cl$_3$; 2, [Co(NH$_3$)$_4$H$_2$O]$_2$(SO$_4$)$_3$; 3, [Co(NH$_3$)$_5$Cl]Cl$_2$; 4, [Co(NH$_3$)$_3$(C$_2$O$_4$H)](NO$_3$)$_2$; 5, [Co(NH$_3$)$_4$CO$_3$]NO$_3$; 6, trans-[Co(en)$_2$Cl$_2$]Cl, using 1 mM CSA as running electrolyte. Voltage applied, 20 kV. Detection at 210 nm. Peak 7 corresponds to electroosmotic flow boundary.
Fig. 5
Electropherograms obtained using 1 mM CSA containing 1 mM BTAC without (A) and with (B) the addition of 10 mM β-cyclodextrin as running electrolytes for a mixture containing 25 mg L$^{-1}$ trimethyltin chloride (1), 15 mg L$^{-1}$ triethyltin chloride (2) and 20 mg L$^{-1}$ tributyltin chloride (3). Voltage applied, 20 kV. Detection at 218 nm.
Fig. 6
Electropherogram obtained from a mixture of triorganotin compounds using as running electrolyte a 20 mM solution of tartaric acid in 4:1 water-methanol containing 4 mM BTAC. Applied voltage, 20 kV. Detection at 218 nm. The sample injected was obtained by 500-fold preconcentration of a mixture containing 2 µg L⁻¹ trimethyl-, triethyl- and tributyltin chlorides (peaks 1, 2 and 3, respectively) and 0.2 µg L⁻¹ triphenyltin chloride (peak 4) on Amberlite XAD-2 and elution with methanol.
Table I

Efficiency of preconcentration of TBT and TPT from natural water samples (1 L) spiked with 2 µg L\(^{-1}\) TBT and 0.2 µg L\(^{-1}\) TPT. Solid-phase extraction was performed on 0.5 g XAD-2. The compounds were eluted with 10 mL methanol which was evaporated to 2 mL before injection.

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<tr>
<th>Sample</th>
<th>Recovery of triorganotin compounds (%)</th>
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<tr>
<td></td>
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REFERENCES


