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Speciation of heavy metals by capillary electrophoresis☆

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Abstract

The general abilities of capillary electrophoretic methods [capillary zone electrophoresis (CZE), isotachophoresis and isoelectric focusing] to perform species analysis are described. Several examples of the speciation of heavy metal complexes (e.g., complexes of gadolinium, platinum and iron) and metal oxoanions (arsenic and selenium) using CZE are demonstrated and the applicability to separation problems in the fields of medicine, pharmacology and ecology is discussed.

1. Introduction

In recent years, the level of knowledge about the different chemical forms of the elements has continually increased. The term "speciation" was used in the past only for metals. Today speciation, the determination of the different bonding forms and oxidation states of an element in a sample, which together add up to the total concentration, is applied to the whole Periodic System (excluding C, H and O). The reasons for stepping up species analysis are attributed to the different chemical and biological behaviours of each species. For the general assessment of ecosystems it is essential to know which species of the elements exist in the systems, as they have a major influence on the processes of distribution, accumulation and decomposition. In medicine bioavailability, toxicity, essentiality or enzymatic principles of action are determined by the interaction of different species in the biological material. All species possess their own characteristic behaviour and can react in different matrices to different reaction products. Therefore, high demands are placed on analytical methods and sample preparation to determine all bonding forms of a particular element in a sample simultaneously, as the species can possess very similar in addition to very different chemical and physical properties. Most analytical methods are not applicable to a direct species analysis, because the resolving power of the technique sets limits for the differentiation between very similar species or the species are transformed irreproducibly during the analysis. In Table 1 an overview is given of the few analytical methods that can be used for direct speciation. If an additional separation step is integrated into the analysis, speciation is also possible using a number of common analytical methods (Table 2). Among them, capillary elec-

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Table 1
Analytical methods for direct speciation measurements

Analytical method		Detection parameter
AES	Auger electron spectroscopy	E of Auger electrons
XPS	X-ray photoelectron spectroscopy	E of photoelectrons
EXAFS	Energy-dispersive X-ray absorption fine structure	Kα
ESR	Electron spin resonance spectroscopy	E of resonance absorption
NMR	Nuclear magnetic resonance spectroscopy of the nuclei ^{19}F, ^{25}Mg, ^{27}Al, ^{29}Ai, ^{31}P, ^{43}Ca, ^{113}Cd, ^{195}Pt, ^{199}Hg and ^{207}Pb	ν frequency of resonance
Mössbauer spectroscopy	Mass spectrometry	m/z
Electrochemistry	Amperometry, polarography	U^0

trophoresis is characterized by the highest resolving power and extensive possibilities of varying the analytical conditions. The separation process is performed on the basis of the different electrophoretic mobilities, which depend on the structures and the radii of the ions, therefore possessing ion-specific characteristics. Even very similar species with nearly the same electrophoretic mobilities (e.g., differences of 0.05% between two electrophoretic mobilities) can be separated by this method and the preservation of the original bonding forms is often possible by choosing suitable buffer systems. An overview of the few papers dealing with direct speciation through electrophoretic separations in capillaries is given in Table 3.

In this work, we tested the potential of capillary zone electrophoresis (CZE) for speciation measurements using different kinds of samples, especially pharmaceutical formulations. We demonstrate the ability of the method to separate metal oxoanions (species of arsenic and selenium) with very different and metal complexes (species of platinum, gadolinium and iron) with very similar electrophoretic mobilities.

Table 3
Speciation measurements with capillary electrophoresis methods (CZE, ITP, IEF)

Species	Method used	Ref.
$[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$	ITP	1
$[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$	CZE	2
SeO_3^{2-}/SeO_4^{2-}, TeO_3^{2-}/TeO_4^{2-}	ITP	3
Aluminium fluoride and oxalate	CZE	4
Organic selenium and lead compounds	CZE	5
Cobaltocarboranes	ITP	6
Cobalamines	CZE, HPLC	7, 8
Haemoglobins	IEF, CZE	9, 10
AsO_3^{3-}/AsO_4^{3-}	CZE	11

Table 2
Analytical methods for direct speciation after a separation step

Analytical method	Specific property used for separation	Detection parameter
HPLC (HPLC–AAS or HPLC–ICP-AES)	Distribution, adsorption, ion exchange, steric exclusion	λ of atomic absorption and emission lines
GC (GC–AES or GC–MS)	Distribution, adsorption	λ of atomic emission lines, m/z
Ion chromatography	Distribution, ion exchange	pK_a, conductivity
Electrophoresis (IEF, ITP, CZE)	Electrophoretic mobility, pI	UV–vis absorption, fluorescence emission, conductivity

2. Experimental

2.1. Materials

All phosphate and chromate buffers were prepared with sodium salts of research grade (Merck, Darmstadt, Germany). Methanol (LiChrosolv for chromatography) (Merck) and acetonitrile (R Chromasolv for HPLC) (Riedel-de Haën, Seelze, Germany) were used for the addition to the separation buffers. To adjust the pH of the buffer, tris(hydroxymethyl)aminomethane(Tris) and sodium hydroxide (both from Merck) were used. Hexadecyltrimethylammonium bromide (HTAB) (Merck) was used as an osmotic flow modifier and sodium dodecyl sulfate (SDS) (Riedel-de Haën) as a micelle-forming agent.

For the preparation of standard solutions of different metal-containing species, the following substances (all of research grade) were employed: $NaAsO_2$ (solution), H_3AsO_4, NaCl, Na_2SO_4 (all from Merck), $Na_2SeO_3 \cdot 5H_2O$, Na_2SeO_4 (Fluka, Buchs, Switzerland) and ferrocene, ferrocenecarboxaldehyde, butylferrocene, $Gd(Cl)_3 \cdot 6H_2O$ and diethylenetriaminepentaacetic acid (all from Aldrich, Steinheim, Germany). The ligand for the Gd complex Roman 1 was synthesized according to a new procedure [12]. Rat serum and lobaplatin [1, 2 - di(aminomethyl)cyclobutaneplatinum(II) lactate] were supplied by Arzneimittelwerk Dresden (Dresden, Germany).

2.2. Apparatus

A P/ACE 2000 capillary electrophoresis system from Beckman (Palo Alto, CA, USA) was used for CZE measurements. In all measurements the UV–Vis absorption of the double or triple bonds in the organic part of the analyte compounds or the UV signals of the buffer systems were utilized for the detection of the species at 200, 214 and 254 nm using a detector system with fixed wavelengths. Polyimide-clad fused-silica capillary tubing of 50 and 75 μm I.D. was obtained from CS-Chromatographie Service (Langerwehe, Germany).

3. Results and discussion

3.1. Species of arsenic and selenium

The inorganic species arsenite, arsenate, selenite and selenate were the first to be subjected to species analysis. Several papers have dealt with the simultaneous determination of both oxidation states of these elements [3,11,13] and of their organometallic compounds [14,15].

In aqueous solution the species exist as small oxoanions, which possess one to three negative charges depending on the pH of the electrophoresis buffer. Therefore, they rapidly migrate to the anode and can be separated in the same buffers such as chloride, sulfate or carbonate. Because of the low absorptivity in the UV region, a UV-absorbing buffer such as chromate, which must migrate fairly fast, is to be preferred. As can be seen from Table 4, the anions in the higher oxidation states, arsenate and selenate, possess very low $pK_{a,1}$ values and even at medium pH have two negative charges. Arsenate and selenate will migrate very fast over a wide pH range of 6.5–10. Arsenite and selenite show a higher charge density on the surface because of smaller ionic radii and therefore the dissociation of protons makes higher demands on energy consumption. Hence the $pK_{a,1}$ values are high and both oxoanions possess only a negative charge of <-1. Large differences in charge at medium pH are not useful for fast speciation, because the slowly migrating species arsenite and selenite give broad peak shapes and the analysis time is very long. If only selenite and selenate have to be separated, a pH of 9 is sufficiently high for a fast separation. Fig. 1 shows the separation of all four species operating at pH 10.

Table 4
pK_a values of arsenic and selenium acids

Acid	$pK_{a,1}$	$pK_{a,2}$	$pK_{a,3}$
H_3AsO_4	2.18	6.77	11.23
H_3AsO_3	9.40	13.52	
H_2SeO_4	<0	1.92	
H_2SeO_3	2.54	8.02	

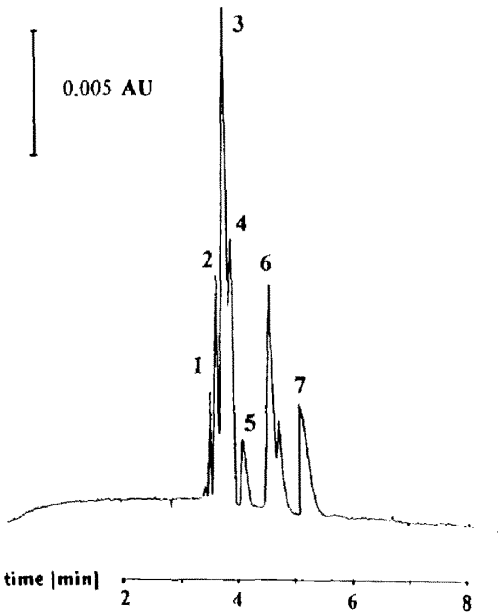

Fig. 1. Separation of arsenic and selenium species. Buffer, 5 m*M* chromate (pH 10.0, adjusted with Tris)–0.25 m*M* HTAB; capillary, 50/57 cm × 75 μm I.D.; $U = -15$ kV; indirect detection at 254 nm. Pressure injection of a 10^{-4} *M* solution of each component. Peaks: 1 = Cl^-; 2 = SO_4^{2-}, 3 = $HAsO_4^{2-}$; 4 = SeO_4^{2-}; 5 = F^-; 6 = SeO_3^{2-}; 7 = $H_2AsO_3^-$.

Only at this pH is speciation of arsenite and arsenate possible in the chromate buffer system. For a good separation of all compounds in this sample, measurements at reversed polarity and with the addition of an osmotic flow modifier (OFM), such as HTAB, is necessary. Depending on the concentration of this modifier the negative charge of the capillary wall, generated by the dissociation of silanol groups, is decreased, removed or reversed. In this way the separation time can be shortened by elimination or reversal of the electroosmotic flow.

3.2. Complexes of gadolinium

There is a continuing search for suitable paramagnetic compounds usable as contrasting agents in magnetic resonance imaging. Gadolinium–diethylenetriaminepentaacetic acid complex (Gd–DTPA) possesses the most suitable characteristics of high magnetic moment, high stability ($\log K = 23$) and low toxicity. Because of the high toxicity of free Gd^{3+} ion, the determination of the dissociation behaviour of the complex in vivo and the separation of the Gd–DTPA complex from other gadolinium complexes is required.

A complete separation of all three components, Gd–DTPA, free DTPA ligand and free Gd^{3+} ion, has not previously been successful because of the very different chemical properties of the three components and their poor absorption characteristics in the UV–Vis region. Separation of the complex and the ligand was achieved by Vora et al. [16] using HPLC. In our work we tried to separate Gd–DTPA from another gadolinium complex, which was formed with a new polyaminopolyacetic acid ligand, synthesized after a recently developed procedure [12] and currently the subject of suitability tests

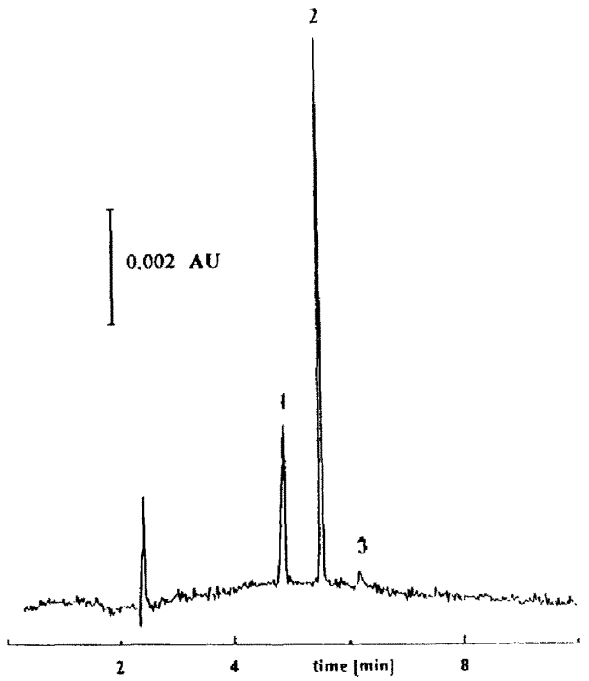

Fig. 2. Separation of Gd complexes. Buffer, 25 m*M* phosphate (pH 6.8)–10% acetonitrile; capillary, 50/57 cm × 50 μm I.D.; $U = 25$ kV; $I = 60$ μA; detection at 200 nm. Pressure injection of a mixture of Gd–DTPA complex and Roman 1 (Gd complex), each complex 10^{-3} *M*. Peaks: 1 = Roman 1 (Gd complex); 2 = Gd–DTPA complex; 3 = impurity from 2.

for magnetic resonance imaging. Both complexes possess high stability constants, and therefore a separation in an electrophoretic buffer is possible. The chosen separation conditions, demonstrated in Fig. 2, allow a very fast separation of both complexes, although the free ligands could not be detected. The free ligands are highly dissociated and because of the absence of ion-pairing compounds in the buffer the negative charges could not be compensated. The hydrated free Gd^{3+} moves very fast. Nevertheless, the detection of the ion was impossible because of its very low molar absorptivity in the detectable UV region.

The demonstrated separation is suitable for the determination of complex stabilities and will be used for the characterization of newly synthesized polyaminopolyacetic acids as ligands for gadolinium complexes in comparison with the DTPA ligand.

3.3. Separation of diastereomeric platinum complexes

Platinum complexes have been successfully applied in cancer therapy for many years [17]. Well known is cisplatin [*cis*-diamminedichloroplatinum(II)] as the first compound to be administered. Second-generation substances are iproplatin [*cis*-dichloro-*trans*-dihydroxydi(isopropylamine)platinum(IV)] and lobaplatin [1,2-di(aminomethyl)cyclobutaneplatinum(II) lactate], which are now undergoing clinical tests.

Lobaplatin is a racemic mixture of diastereomers. To control the efficiency of dosages given to patients, separation of the diastereomers in

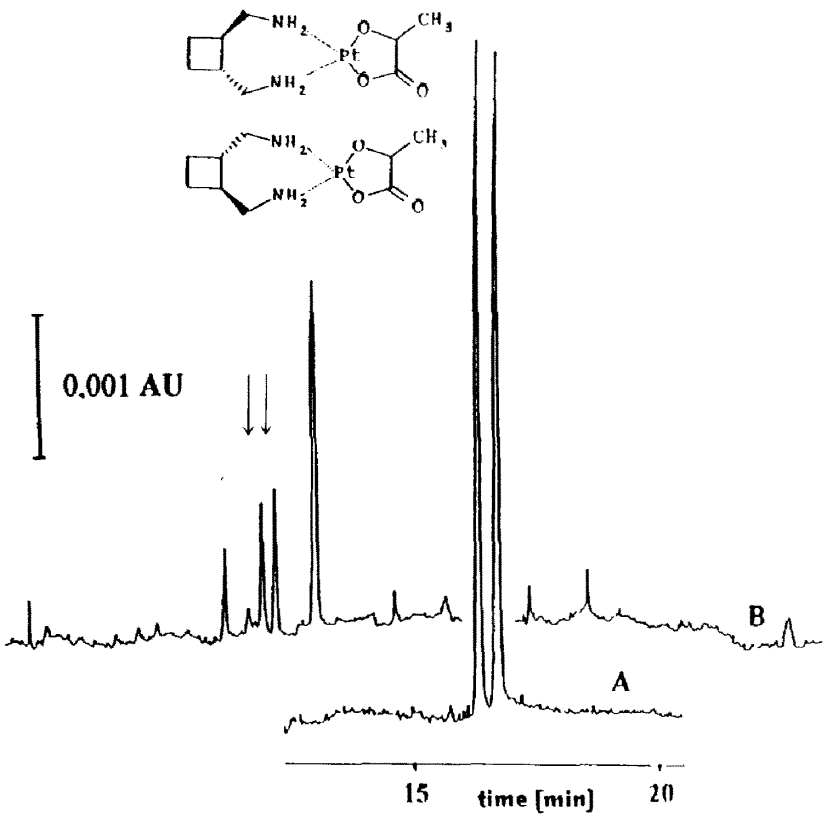

Fig. 3. Separation of diastereomeric platinum complexes (lobaplatin). Buffer, 20 m*M* phosphate (pH 6.7)–(90 m*M* SDS; capillary, 70/77 cm × 75 μm I.D.; U = 20 kV; I = 190 μA; detection at 214 nm. (A) Standard solution ($5 \cdot 10^{-4}$ *M*) of platinum complexes; (B) rat serum (diluted 1:4 with water), spiked with platinum complexes.

plasma is necessary. In Fig. 3A a lobaplatin standard containing $2.82 \cdot 10^{-4}$ *M* of each diastereomer was separated. The buffer was chosen considering the pH range of real samples. Various concentrations of the micelle-forming agent SDS were applied to optimize the separation. The addition of micelle-forming compounds to the buffer leads to the introduction of supplementary separation effects, comparable to the interaction of an analyte with the stationary phase in liquid chromatography and therefore called a "quasi-stationary phase" [18]. Hydrophobic components are able to interact inside the micelles, which also possess a hydrophobic character. Differences in the partition coefficients between the buffer and the micelles, existing for two compounds with nearly the same electrophoretic mobilities, result in different migration times owing to the electrophoretic migration of the micelles. Advantage can be taken of this effect for the separation of diastereomers. With increasing amount of added SDS an increase in resolution of the diastereomers was achieved, explainable by the different partition coefficients for the two diastereomers inside the micelles. Fig. 3A represents an optimum separation concerning resolution and analysis time. In Fig. 3B, prior to the analysis lobaplatin was adjusted to a concentration of $1.41 \cdot 10^{-4}$ *M* for each diastereomer in the rat plasma. In addition to lobaplatin diastereomers some proteins of the plasma could also be separated. Reproducible repetitions of this analysis are difficult to achieve, however, because of protein adsorption on uncoated capillary walls. Unfortunately, under the chosen analytical conditions without a preconcentration step the detection limit for platinum compounds is not low enough for a real plasma analysis because of the relatively low absorptivity of the compounds and the apparatively fixed detection volumes.

3.4. *Ferrocene and ferrocene derivatives*

Ferrocenes are characterized by wide applicability in the modern industry [19], especially as catalysts and photosensitizers in the organometallic and semiconductor industry, and in medicine as anticancer drugs. We developed a separation method for ferrocenes to control the purity of commercially available and newly synthesized compounds and to investigate degradation processes of these substances.

In Fig. 4, the separation of three purchased ferrocenes is demonstrated. To avoid recrystallization of the dissolved ferrocenes inside the capillary, the application of a buffer containing large amounts of acetonitrile was essential. At the applied buffer pH all three compounds possess a net charge of zero, and therefore the differences in their migration should be reduced to the interaction inside the micelles of the SDS molecules. For butylferrocene we were able to confirm the specification of a content of 97% certified by the manufacturer.

Fig. 5 shows the separation of the synthesized ferrocene derivatives [20]. These compounds are candidates for application as mediators in scan-

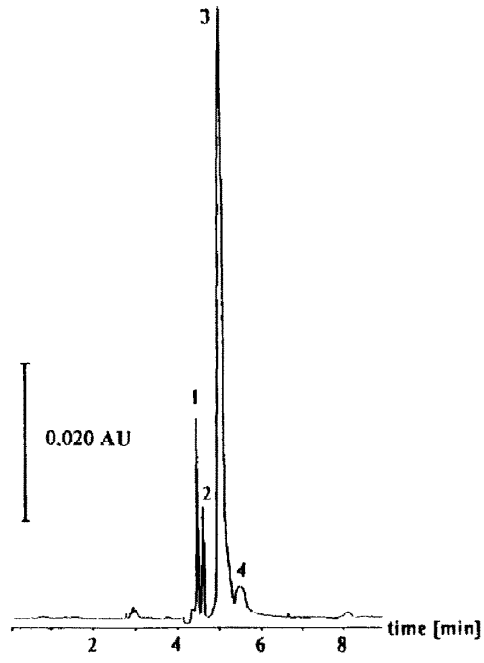

Fig. 4. Separation of ferrocene derivatives. Buffer, 10 m*M* phosphate (pH 6.7)–30 m*M* SDS–35% acetonitrile; capillary, 70/77 cm × 75 μm I.D.; *U* = 25 kV; *I* = 86 μA; detection at 214 nm. Peaks: 1 = ferrocenecarboxaldehyde; 2 = ferrocene; 3 = butylferrocene; 4 = impurity from 3.

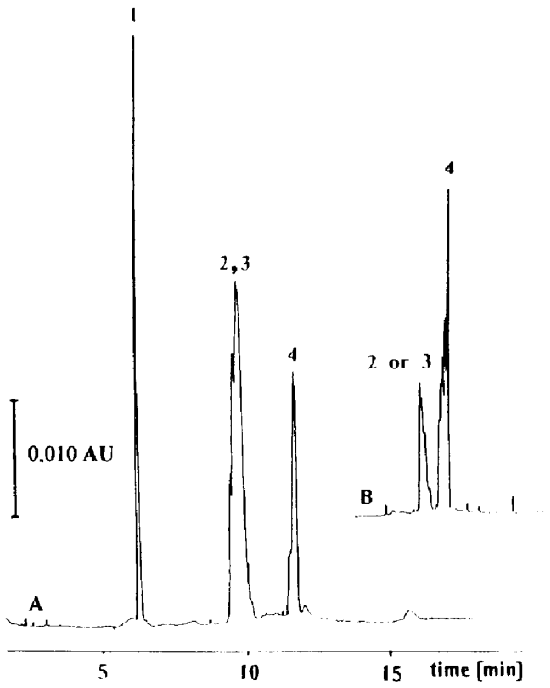

Fig. 5. Separation of ferrocene derivatives. Buffer, 10 m*M* phosphate (pH 6.6)–30 m*M* SDS–35% acetonitrile; capillary, 70/77 cm × 75 μm I.D.; *U* = 30 kV; *I* = 72 μA; detection at 214 nm; pressure injection. Peaks: 1 = (trimethylaminomethyl)ferrocene; 2 = (dimethyl-*n*-dodecylaminomethyl)ferrocene (*n* = 5); 3 = (dimethyl-*n*-hexadecylaminomethyl)ferrocene (*n* = 7); 4 = (dimethyl-*n*-octylaminomethyl)ferrocene (*n* = 3). (A) Mixture of all four ferrocene derivatives; (B) (dimethyl-*n*-octylaminomethyl)ferrocene.

ning electrochemical microscopy and for the surface modification of electrodes for electrochemical measurements, where the expected signals should depend on the chain length of the fixed ferrocenes. The separation of all four synthesized derivatives (Fig. 5A) was not completely achieved, only the two compounds with the shortest chain length are well separated. The observed migration order is not typical of CZE or even HPLC, where in most instances a proportionality between chain length and migration/retention time is found. It appears that the synthesis of the compounds giving peaks 2 and 3 did not produce the expected compounds. This assumption was also confirmed by NMR measurements which were performed later. In Fig. 5B the separation of (dimethyl-*n*-octylaminomethyl)ferrocene shows that even during this synthesis byproducts were formed. We found that the alkyl bromides purchased for the synthesis were mixtures of bromides with different lengths of the alkyl chains. Further investigations are now directed to synthesizing pure derivatives usable for analytical purposes.

Because of its resolving power, CZE is well suited to separate different species of one element simultaneously with both nearly the same and very different electrophoretic mobilities. In the future efforts need to be made to achieve lower detection limits for species with low molar absorptivities in the UV–Vis region utilizing preconcentration steps or suitable detection systems.

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