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Analysis of metal species by using electrospray ionization mass spectrometry and capillary electrophoresis–electrospray ionization mass spectrometry

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Abstract

Metal speciation was carried out by on-line hyphenation of capillary electrophoresis (CE) with mass spectrometry (MS) via an electrospray ionization (ESI) interface. The commercially available interface was hardly able to produce stable electrospray conditions over an extended period of time, mainly caused by an insufficient positioning of the CE capillary inside the ESI stainless steel tip. A device was developed, which allowed an infinitely variable adjustment of the capillary. The optimum position for stable electrospray conditions was set to 0.4–0.7 mm outside the ESI tip. Off-line ESI-MS investigations of free metal ions [Cu(II)], metal ion-containing complexes [CuEDTA, $(CH_3)_3SbCl_2$] and covalent organometallic compounds (selenocystamine, selenomethionine) were carried out in order to assess the suitability of the technique for metal speciation. The usefulness of ESI-MS as a detection method largely depends on the stability of the analysed species. Inorganic species (i.e. metal ions) alter their composition when being electrosprayed. Parts of the weakly complexing ligands will be exchanged by solvent molecules, mainly originating from the sheath liquid. The destruction of ion–solvent clusters by heating, collision-induced decomposition or use of a sheath gas may lead to charge reduction of transition metal ions. Organometallic complexes with strongly complexing ligands remain intact, while those with weakly complexing ligands suffer from the same disadvantages as inorganic species. ESI-MS is best suited for the speciation of covalent organometallic compounds. The ionisation process does not alter their structure and they will mostly be detected as singly charged molecular ions. The application of CE–ESI-MS for selenium speciation to an existing method using an alkaline buffer system (Na_2CO_3–NaOH) gave unsatisfactory results. The non-volatile electrolyte affects the ESI process dramatically. The final CE method used an acidic background electrolyte (2% acetic acid) for the separation of three organometallic selenium species [selenomethionine (SeM), selenocystamine (SeCM) and selenocystine (SeC)]. The Se species were sufficiently separated from each other and appeared at 6.49 min (SeCM), 19.47 min (SeM) and 20.60 min (SeC). Detection limits were calculated as 1–6 mg/l for the organic Se species.

Keywords: Interfaces; CE–MS; Metals; Organoselenium compounds; Copper; Selenium

1. Introduction

The term (chemical) speciation, according to the EU (M&T Program, formerly BCR) and IUPAC has been defined as the clear identification and quantification of a species (specific compound or oxidation state of an element) in a real sample/matrix [1]. In the case of organometallic complexes the organic bonding/complexing partners have to be taken into account as well [2]. In order to assess the biological

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and (eco)toxicological behaviour of an element, the knowledge of its chemical species is absolutely necessary. Therefore, powerful analytical techniques are required for speciation analysis.

The coupling of capillary electrophoresis (CE) and mass spectrometry (MS) combines the advantages of CE and MS so that information on both high separation efficiency and molecular masses and/or fragmentation can be obtained in one analysis. Analytes are distinguished by both their differences in electrophoretic mobilities and structural information. Since CE separations occur in the liquid phase and MS detection is a gas-phase process, electrospray (ESI) or ion spray (IS), the pneumatically assisted version of electrospray, are particularly suitable as interfacing techniques, since these atmospheric pressure ionization (API) techniques produce gas-phase analyte ions directly from solution [3–8]. In this regard, the ESI interface with a coaxial sheath liquid arrangement is reported to be very effective [9–11]. However, there are some difficulties to be considered when CE and MS are combined on-line: (i) CE background electrolytes are limited to volatile compounds [12]; (ii) migration times of analytes in a CE–ESI-MS separation can be affected by the sheath liquid composition [13]; and (iii) CE–ESI-MS is lacking in absolute concentration sensitivity. Two reviews [13,14] dealing both with methodical and technical changes in order to improve the concentration sensitivity of CE–MS have been published recently.

CE has been widely applied to the separation of inorganic species as can be found in two review articles [15,16]. The most common detection method for elemental analysis is indirect photometric detection, but recently there have been a number of reports of ESI-MS mass spectra of transition metals-containing species, either as solvated complexes [17,18] or as complexes with peptides–proteins [19,20]. In addition, Huggins and Henion [21], as well as Corr and Anacleto [22], have reported the analysis of both inorganic cations and anions by CE coupled to ion spray. They were able to separate and detect 12 and 29 inorganic cations, respectively.

One aim of the present work was the setting up and optimization of a CE–ESI-MS system providing stable electrospray conditions over an extended period of time. Preliminary investigations showed that the system is a powerful tool for elemental analysis and, therefore, is suited for chemical speciation. But it also turned out that the information obtained by ESI-MS strongly depends on the stability of the analysed species and, for that reason, great care has to be taken on the interpretation of mass spectra. Therefore, prior to hyphenation, extensive off-line ESI-MS investigations on different metal-containing substances (free metal ions, metal ion-containing complexes and covalent organometallic compounds) have been carried out, in order to test the suitability of ESI-MS for metal speciation. The only class of compounds being unrestricted in terms of application for ESI-MS detection are covalent metal organic compounds. In order to demonstrate that, the on-line interfacing of CE and ESI-MS was finally carried out, separating and detecting three selenium-containing species. Several studies on selenium speciation have been done at our laboratories [23,24], mainly using CE–inductively coupled plasma (ICP)-MS. An existing method for selenium speciation by CE–ICP-MS shall be adapted for ESI-MS detection.

2. Experimental

2.1. Chemicals and reagents

Standard stock solutions for SeCM, SeC and SeM (final concentration each 0.5 g/l) as well as a copper(II) stock solution (1 g Cu/l) were prepared by dissolving the appropriate amount of selenocystamine·2HCl, seleno-DL-cystine (both Sigma, Deisenhofen, Germany) and copper(II) chloride dihydrate (Merck, Darmstadt, Germany, analytical-reagent grade) in Milli-Q water. Seleno-DL-methionine (Sigma) was dissolved in 20 m*M* KOH as it is nearly insoluble in pure water. Trimethylantimony dichloride ($TMSbCl_2$) was a donation of Professor W. Cullen, Vancouver, Canada, and was synthesized there according to published methods [25,26]. A standard stock solution (100 mg Sb/l) was prepared by dissolution in water. The appropriate amount of free EDTA was partially dissolved in water in order to get a standard stock solution of 0.25 mol/l. Complete dissolution was achieved by adding a solution of concentrated ammonia (25%)

until the pH was adjusted to 7.0. The copper–EDTA complex (CuEDTA) was prepared by mixing the appropriate amounts of the copper(II) and the EDTA stock solutions. Standard solutions of lower concentrations for ESI-MS optimization were prepared daily by dilution with water. Standard solutions of the three seleno compounds for CE separation were prepared by dilution with CE electrolyte (2% acetic acid, pH 2).

All chemicals used for preparation of samples, electrospray sheath liquids and CE electrolytes were of analytical-reagent grade or higher purity. Water was first purified by normal deionization and then by a special cartridge ion-exchange unit (Milli-Q water purification system).

2.2. Instrumentation

2.2.1. Off-line ESI-MS detection

The system consisted of a triple-quadrupole mass spectrometer TSQ 700 and an electrospray ionization unit (both: Finnigan MAT, Bremen, Germany). Sample introduction was performed using a syringe infusion pump 22 (Harvard Apparatus, South Natick, MA, USA) through an uncoated fused-silica capillary, which was 40 cm×50 μm I.D. in size. Up to 1 cm of the outside polyimide cover was removed from the end of the capillary to aid wetting of the tip by the sheath liquid. The flow-rate of the pump was set to 0.5 μl/min for optimization investigations. A second syringe infusion pump delivered the sheath liquid with a flow-rate of 1.0 μl/min. The capillary sizes were the same as above. Table 1 summarizes the ESI conditions used for positive ion mode investigations of different samples. Data acquisition was performed by the third quadrupole in the full scan mode over the mass range of interest, with two scans per s. The electron multiplier voltage (U_{mult}) was set to 1.65 kV.

Special care was focused on an exact positioning of the capillary. Therefore the BioFocus CE–MS interface kit (Bio-Rad, Munich, Germany) providing temperature control of the capillary close to the electrospray interface and connecting the CE system to the mass spectrometer has to be modified. Fig. 1 shows a scheme of the modified CE–MS interface kit, which allows an infinitely variable adjustment of the capillary in the electrospray interface.

2.2.2. CE–ESI-MS hyphenation

A BioFocus 3000 CE system (Bio-Rad, Munich, Germany) was used as the CE device. In contrast to Section 2.2.1, the sample capillary was replaced by the CE capillary, positioned in the same way. The temperature was set to 20°C for sample carousel (air cooling) and capillary (liquid cooling) during all experiments. The uncoated capillary was UV transparent and 75 cm×50 μm I.D. in size (inlet/UV detection, 20 cm; UV detection/ESI probe tip, 55 cm). Up to 1 cm of the polyimide coating was removed from the end of the CE capillary to aid wetting of the tip by the sheath liquid. UV detection was at 200 nm over a total run time of 30 min.

Preliminary experiments used an alkaline background electrolyte (Na_2CO_3–NaOH; pH 11.5) [27] aiming for deprotonation of Se species. The applied

Table 1
ESI-MS operating conditions

No.	Sample	Sheath liquid composition (v/v)	Spray potential, U_{es} (kV)	Spray current, I_{es} (μA)	Temperature of heated capillary, T_{cap} (°C)
1	SeM (CE–ESI-MS)	MeOH–water–CH_3COOH (85:10:5)	5.4	52–56	200
2	SeM, SeC, SeCM (CE–ESI-MS)	MeOH–water–CH_3COOH (85:10:5)	5.4	52–56	200
3	MeOH–water–CH_3COOH (47.5:47.5:5) (ESI-MS)	MeOH–water–CH_3COOH (47.5:47.5:5)	5.6	53–58	200
4	$CuCl_2 \cdot H_20$ (ESI-MS)	MeOH–water–CH_3COOH (85:10:5)	5.2	50–55	200
5	CuEDTA (ESI-MS)	MeOH–water (85:15)	6.4	62–66	150
6	$TMSbCl_2$ (ESI-MS)	MeOH–water (50:50)	6.4	62–66	100
7	SeCM·2HCl (ESI-MS)	MeOH–water–CH_3COOH (85:10:5)	5.4	52–56	175
8	SeM (ESI-MS)	MeOH–water–CH_3COOH (85:10:5)	5.2	50–54	200

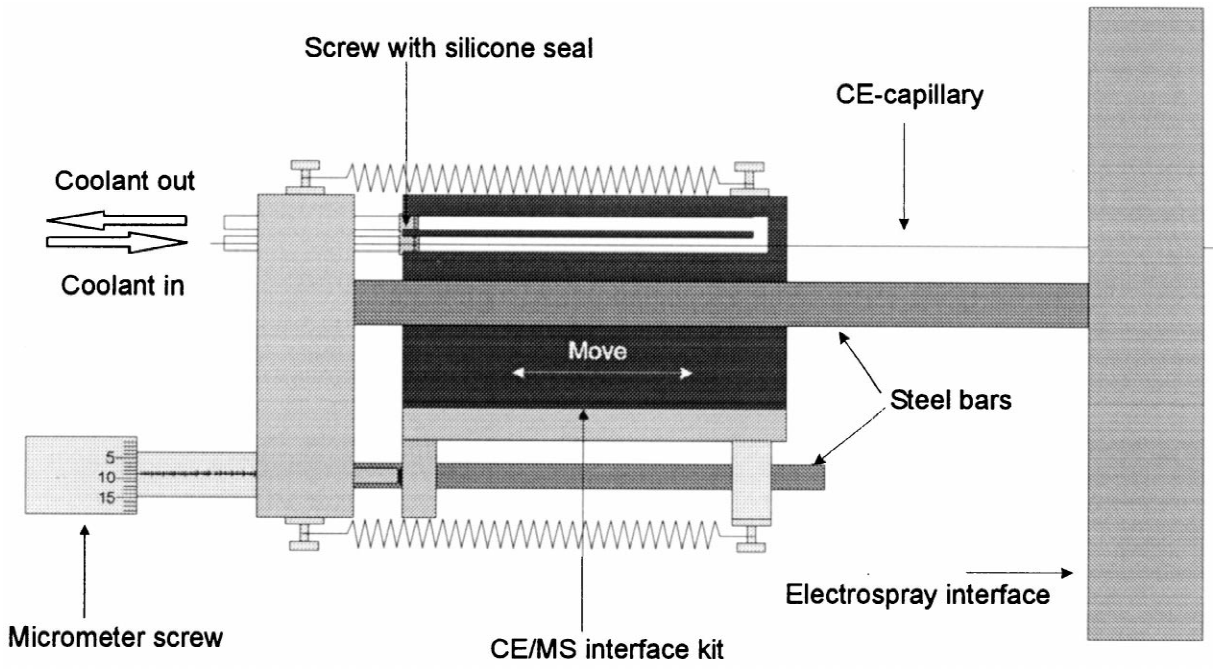

Fig. 1. A scheme of the modified CE–MS interface kit is shown. The kit is moveable, mounted on two stainless steel bars. By turning the micrometer screw forward, the whole CE–MS interface kit moves along the axis of the electrospray interface leading to an expansion of the two springs. As the sample capillary is fixed to the kit by a screw and a silicone seal, it also moves forward. When turning the micrometer screw backwards the springs contract and pull the kit back. In this way, the capillary's position to the nebulization point is optimized.

voltage was set to +18 kV, resulting in an electrophoretic mobility of anions towards the injection point, but also in a faster electroosmotic flow (EOF) towards the detector. Before each run the capillary was purged with Milli-Q water (120 s, 8 bar) and electrolyte (150 s, 8 bar). The finally developed separation method used 2% acetic acid (pH 2) as CE electrolyte in order to protonate the organic Se species (p*K* values higher than 2 [24]). The applied voltage was set to −25 kV, resulting in an electrophoretic mobility of cations towards the ESI tip. Again the capillary was purged with Milli-Q water (120 s, 8 bar) and electrolyte (150 s, 8 bar) before each run.

Preceding sample injection (pressure, 25 psi·s; 1 psi=6894.76 Pa), the ESI unit was switched off and the potential of the ESI needle was held at ground potential by connecting the syringe infusion pump needle electrically to ground. The CE electrical circuit was closed by the coaxial sheath flow (flow-rate, 1 μl/min). For electrospray conditions see Table 1, nos. 1 and 2. The MS was operated in the full scan mode over a mass range from m/z 150 to m/z 400.

3. Results

3.1. Optimization of the CE capillary's position

The relation between capillary position and total ion current is given in Fig. 2. Sample and sheath liquid were of the same composition. For electrospray conditions see Table 1, no. 3. The scan range of the third quadrupole was set to m/z 10–700. Optimal electrospray operation is only possible at a capillary position ranging from 0.5 to 0.7 mm outside the ESI stainless steel tip. A capillary position beyond this range results in a decrease or breakdown of the ion current. At positions between 0.8 and 1.0 mm, the breakdown of the ion current occurs periodically, with shorter intervals for higher values. At distances over 1.0 mm and below 0.1 mm the ion current breakdown is definitive.

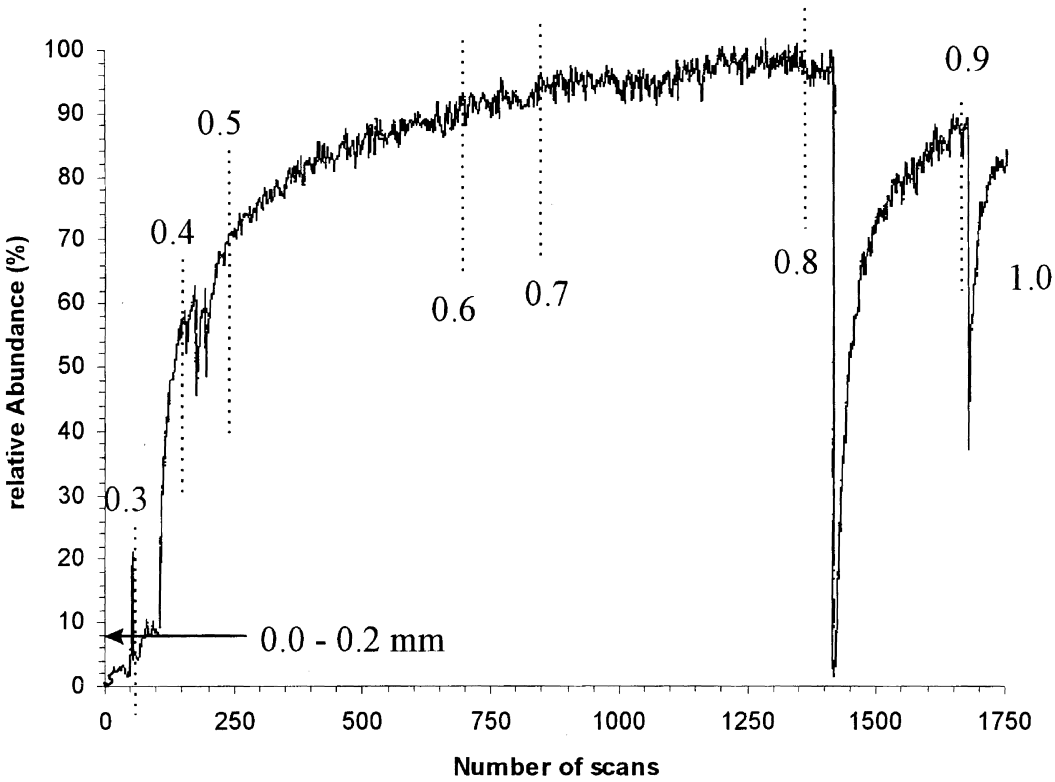

Fig. 2. Relation between capillary position and total ion current. The optimized sample capillary position lies between 0.5 and 0.7 mm outside the ESI stainless steel tip. Other positions lead to insufficient droplet charging causing a weaker signal or a breakdown of the total ion current. For operating conditions see Table 1, no. 3.

3.2. Off-line investigations of metal-containing species

3.2.1. Free metal ions

A mass spectrum of a solution of $CuCl_2 \cdot H_2O$ in water (500 μg Cu/l) is shown in Fig. 3. For operating conditions see Table 1, no. 4. The collision offset (acceleration voltage between skimmer and orifice) for collision-induced decomposition (CID) was set to −25 V, thus being sufficiently high to destroy weak ion–solvent clusters with high mass-to-charge ratios. Additionally to the peaks of the singly charged Cu^+ ion (m/z 63, 65), several ion–solvent clusters are monitored in the mass range up to 160, e.g. $[Cu+H_2O]^+$ and $[Cu+MeOH]^+$. All copper-containing clusters were identified by their isotopic distribution ($^{63}Cu/^{65}Cu$=69.2/30.8%). Increasing the collision offset voltage to −50 V increases the concentration of the Cu^+ peak group to more than 90% (not shown). The application of high temperatures (up to 300°C) on the heated metal capillary has the same effect.

Lowering the collision energy to −10 V, ion–solvent clusters with higher mass-to-charge ratios dominate the mass spectrum (not shown). At least seven different copper-containing peak groups can be identified in the mass range between m/z 120 and 320. The peak group of the singly charged Cu^+ ion is no longer visible.

3.2.2. Metal ion-containing complexes

EDTA forms very stable metal–ligand complexes with Cu(II) (pK=−18.78 for $CuEDTA^{2-}$ [28]). The stabilities of these complexes cannot be suppressed even at high pH. Analysing an aqueous sample containing equimolar amounts of Cu(II) (as $CuCl_2 \cdot H_2O$) and EDTA [as $(NH_4)_2EDTA$] by ESI-MS the following four peak groups can be detected in the

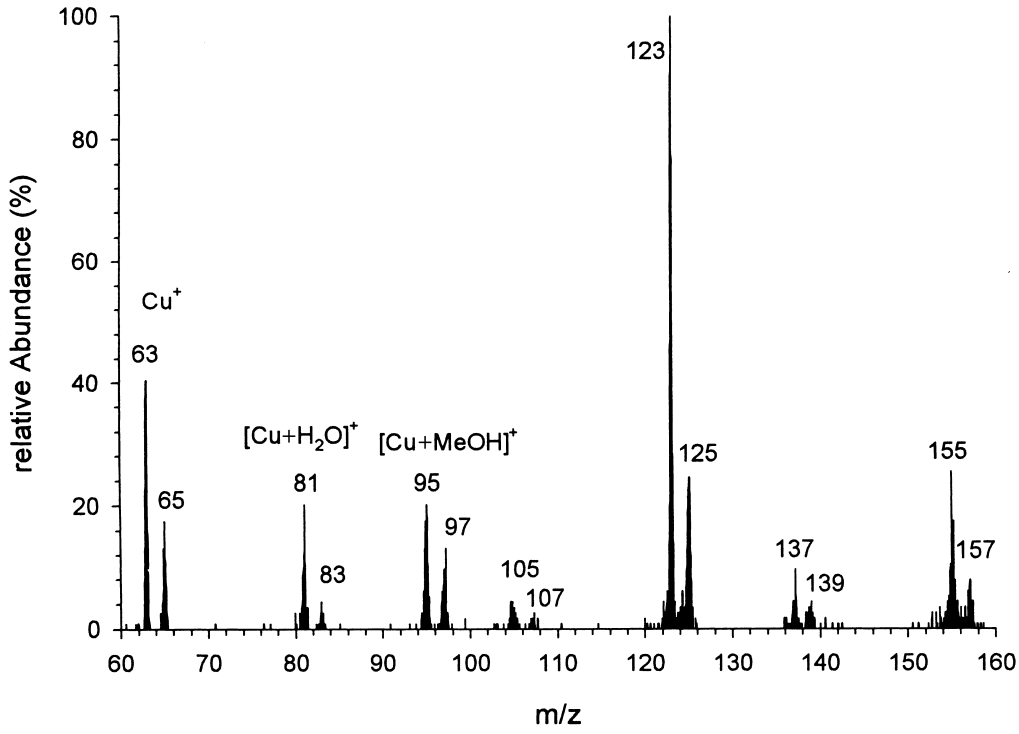

Fig. 3. ESI–MS spectrum of $CuCl_2 \cdot H_2O$ in water 500 μg Cu/l). The collision offset voltage was set to −25 V. For operating conditions see Table 1, no. 4.

positive ion mode (see Fig. 4, for operating conditions see Table 1, no. 5). The final concentration of the sample solution was 0.5 mg/l related to CuEDTA. The main peaks were found at *m/z* 371 and 373 originating from the positively charged ammonia adduct ($[CuEDTA+NH_3+H]^+$), followed by the peaks at *m/z* 390 and 392 corresponding to a species having two water molecules associated ($[CuEDTA+(H_2O)_2+H]^+$) and *m/z* 407 and 409 having two water and one ammonia molecule associated ($[CuEDTA+(H_2O)_2+NH_3+H]^+$). Peaks for the protonated copper–EDTA complex ($[CuEDTA+H]^+$) appear at *m/z* 354 and 356. Spectra were acquired in the full scan mode from *m/z* 300 to 600.

Another example is $TMSbCl_2$. An aqueous sample solution containing 50 mg/l Sb was analysed by ESI-MS using two different sheath liquids [29]. In the presence of acetic acid (sheath liquid, MeOH–water–CH_3COOH, 50:49:1) the $TMSbCl_2$ was detected solely as a positively charged trimethylantimony acetic acid ester ($[(CH_3)_3Sb–OOC–CH_3]^+$), indicated by two peaks at *m/z* 225 and 227 (not shown). Without acetic acid (sheath liquid, MeOH–water, 1:1) various trimethylantimony species were detected (not shown): $[(CH_3)_3SbOH]^+$ (*m/z* 183, 185); $[(CH_3)_3SbOH+H_2O]^+$ (*m/z* 201, 203); $[(CH_3)_3SbCl+MeOH]^+$ (*m/z* 233, 235, 237) and $[(CH_3)_3SbOH+MeOH]^+$ (*m/z* 215, 217). The peak groups were lined up according decreasing intensity. Peaks at 201 and 203 corresponding to $[(CH_3)_3SbOH+H_2O]^+$, together with the peak at mass 205 may also result from the trimethylantimony chloride ($[(CH_3)_3SbCl]^+$), but the mass ratios do not correspond with the isotopic pattern of this compound. For operating conditions see Table 1, no. 6.

3.2.3. Covalent organometallic compounds

An element which is very suited for ESI-MS analysis due to formation of relatively small or-

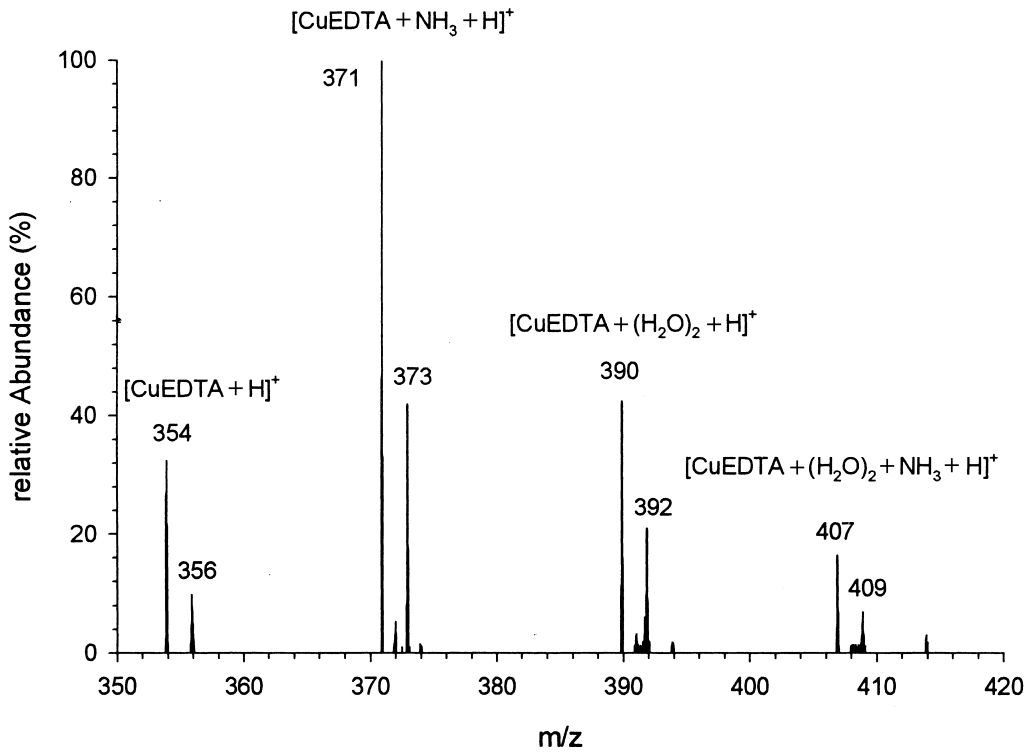

Fig. 4. ESI–MS spectrum of an aqueous solution of equimolar amounts of Cu(II) (as $CuCl_2 \cdot H_2O$) and EDTA [as $(NH_4)_2$EDTA] at pH 7.0. For operating conditions see Table 1, no. 5.

ganometallic compounds is selenium. Electrospray ionization of an aqueous solution of selenocystamine·2HCl (SeCM, 100 mg Se/l) provides a simple mass spectrum (Fig. 5, for operating conditions see Table 1, no. 7). The spectrum is dominated by the protonated selenocystamine $[SeCM+H]^+$, indicated theoretically by 19 peaks between m/z 237 and m/z 255. These peaks are caused by the natural isotopic distribution of selenium: ^{74}Se (0.9%), ^{76}Se (9.0%), ^{77}Se (7.6%), ^{78}Se (23.6%), ^{80}Se (49.7%) and ^{82}Se (9.2%) (natural isotope abundance in brackets). However, some of the 19 peaks cannot be experimentally monitored, because their natural probability lies in the promille range. No ion–solvent clusters can be detected over the whole mass–range independent of the temperature of the heated capillary. A second peak group with a significantly lower intensity appears around m/z 202, belonging to a thermal decomposition product of selenocystamine. This product results from breaking one of the two Se–C bonds. Increasing the temperature of the heated capillary increases the amount of the thermal decomposition product of selenocystamine as well.

Another example of naturally occurring Se species is selenomethionine (SeM, 100 mg Se/l), which has only one selenium atom per molecule (not shown). The spectrum of the electrosprayed basic solution (for operating conditions, see Table 1, no. 8) is dominated by a peak group around m/z 198 originating from the positively charged selenomethionine $[SeM+H]^+$, followed by a peak group around m/z 395 corresponding to the positively charged dimer of selenomethionine $[SeM+SeM+H]^+$. Decreasing temperature to 100°C causes the formation of ion–solvent clusters with methanol and acetic acid ($[SeCM+MeOH+H]^+$, $[SeCM+CH_3COOH+H]^+$,

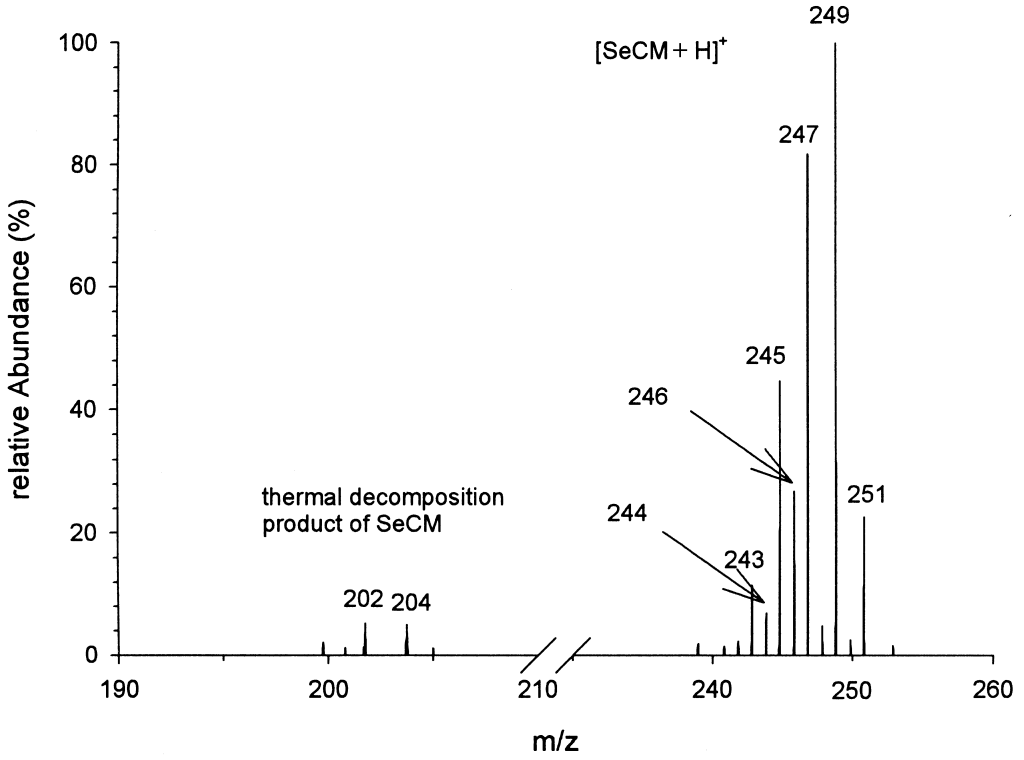

Fig. 5. ESI–MS spectrum of an aqueous solution of selenocystamine + 2HCl (SeCM, 100 mg/l). For operational parameters see Table 1, no. 7.

$[SeCM+CH_3COOH+MeOH+H]^+$, $[SeCM+(CH_3COOH)_2+H]^+$.

3.3. On-line CE–ESI-MS hyphenation for selenium speciation

The analysis of a single standard solution of selenomethionine (SeM) (0.5 g/l) using an existing CE method for the separation of six selenium species [27], gave a sharp UV but a very weak MS signal (not shown, for operating conditions see Table 1, no. 1). In contrast to former investigations with CE–ICP-MS this method was less suitable for CE–ESI-MS. Due to the presence of sodium ions from the alkaline background electrolyte (Na_2CO_3–NaOH) three mass peaks were detected: $[^{80}SeM+Na]^+$ (m/z 220), $[^{80}SeM+2Na]^+$ (m/z 242) and $[^{80}SeM+H]^+$ (m/z 198). The peak groups were lined up according decreasing intensity. The detection limit (3σ of background) for $[^{80}SeM+Na]^+$ was determined at 40 mg SeM/l, the others were even worse. The repetition of analysis led to a shift in the migration time and to a decrease of peak intensities.

However, the method using 2% acetic acid as CE electrolyte and considered less suitable for CE–ICP-MS turned out to be optimal for ESI. MS sensitivity was improved and the results for the separation of the three organometallic selenium species are given in Fig. 6 (for operating conditions see Table 1, no. 2). The concentrations were 25 mg/l for selenomethionine and selenocystamine, and 12.5 mg/l for selenocystine. Separation observed by UV detection seemed to be unsatisfactory (broad, not baseline separated), but ESI-MS detection yielded baseline separated peaks. Peaks appeared at 6.49 min (m/z 247, $[SeCM+H]^+$), 19.47 min (m/z 196, $[SeM+H]^+$) and 20.60 min (m/z 335, $[SeC+H]^+$). Detection limits (3σ of background) were determined at 5.5 mg/l for SeCM, ca. 1 mg/l for SeM and 2.4 mg/l for SeC.

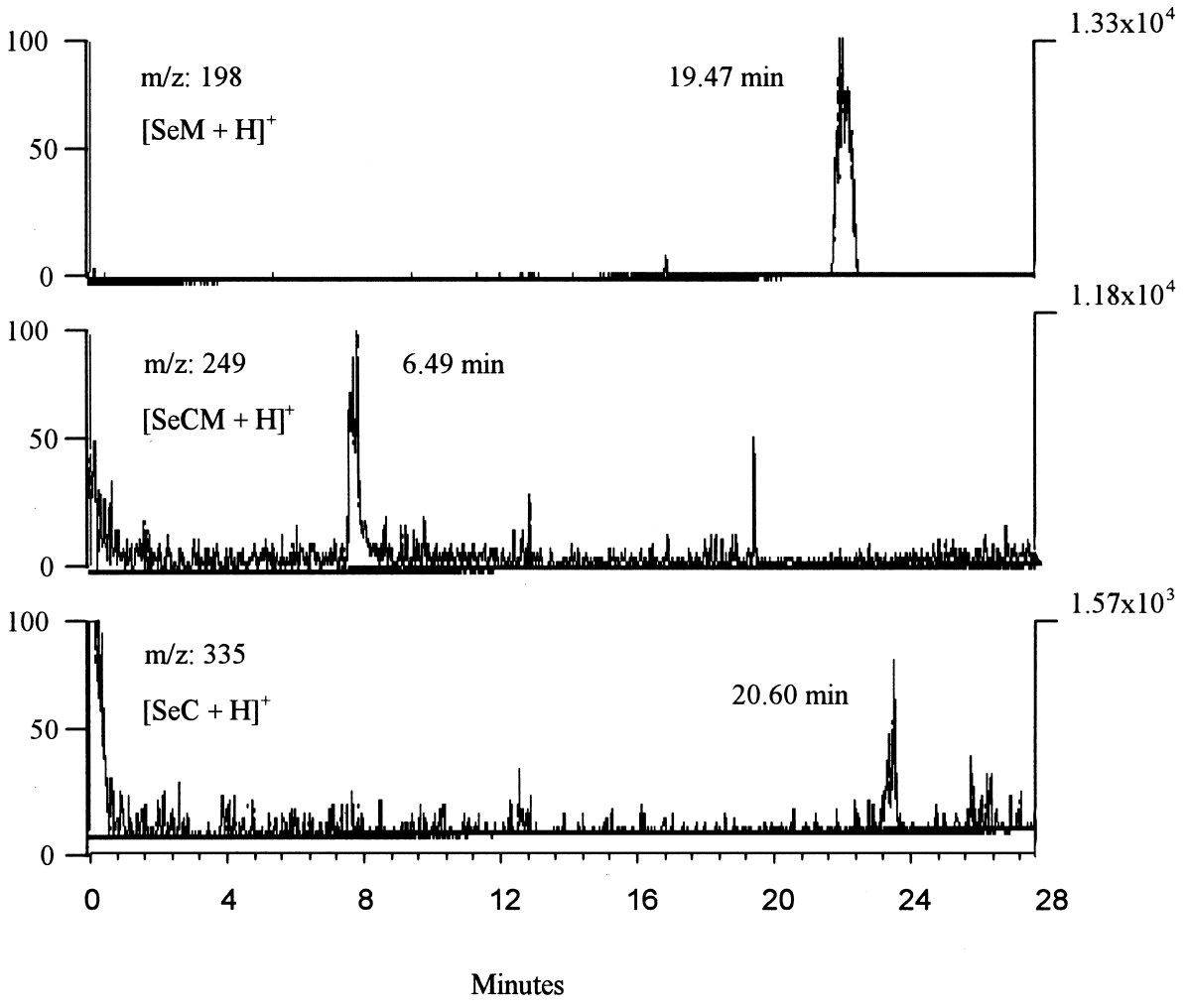

Fig. 6. CE–ESI-MS electropherogram for the analysis of three selenium species (SeM, SeC and SeCM) using 2% acetic acid as CE electrolyte buffer. For operating conditions see Table 1, no. 2.

4. Discussion

4.1. Optimization of the CE capillary's position

As can be seen in Fig. 2, the position of the CE capillary inside the ESI stainless steel tip is very important for stable ESI-MS operation. There are two reasons for this: firstly, the position of the capillary relative to the ESI sheath liquid needle is critical for good electrical contact between CE and ESI; secondly, as the electric field between the ESI stainless steel tip and the heated capillary (counter electrode) is spatially limited, the position of the capillary tip in relation to the position of the electric field is responsible for the extent of droplet charging. If the capillary tip lies in the maximum of the electric field strength (0.4–0.7 mm), the sample liquid disperses into a very fine mist of droplets due to sufficient charging of the surface. The solvent evaporates and most of the droplets reach the critical evaporation limit at which ions are ejected into the gas phase. This results in a high total ion current. If the initial droplet charge is too low to allow a small enough final radius to be achieved, one part of the

droplets do not reach the critical evaporation limit, as can be seen for positions over 1.0 mm and below 0.1 mm. That means that the net charge will simply remain on the particle and no gas phase ions will be observed.

4.2. Speciation of metal-containing compounds

4.2.1. Free metal ions

As can be seen from Fig. 3, the mass spectrum of an electrosprayed aqueous solution of Cu(II) consists of a variety of different copper-containing compounds. Depending on the operating parameters of the MS (mainly collision offset voltage and temperature of the heated metal capillary), several ion–solvent clusters will be formed in the differentially pumped stage between the ion source and the MS [30,31]. The process of ion–solvent cluster formation leads to some potential limitations in the speciation of Cu(II) by ESI-MS.

(i) The process of electrospraying affects the composition of the original species. The weakly complexing chloride ligands of $CuCl_2$ will be partly exchanged by solvent molecules (MeOH, H_2O) forming new species that were not present in the sample solution (e.g. $[Cu+MeOH]^+$). A distinction between ions, that are formed as a consequence of gas-phase ligand replacement reactions and ions, that may arise in ESI by a direct desorption process is not possible at this point in time.

(ii) The destruction of copper(II)–solvent clusters by CID may alter the charging state of the species, as can be seen in Fig. 3. Increasing the collision offset voltage from −10 to −25 V produces more energetic collisions between the copper-adduct molecules and solvent or other gas molecules. The provided spectrum contains the singly charged bare element Cu^+ besides several ion–solvent clusters. The divalent solution ion Cu^{2+} will initially retain its double charged state. As a result of solvent evaporation and insufficient solvent energy, however, the metal ion is unable to maintain the 2+ state. Charge reduction of the cluster is the end result and is depicted by [32,33]:

$$[Cu + (MeOH)_n]^{2+} \rightarrow [Cu + MeO + (MeOH)_{n-2}]^+ + [MeOH + H]^+$$

An application of high temperatures (300°C) to the heated metal capillary has the same effect, as described in the results.

(iii) The detection limit of a species suffers from ion–solvent clustering. According to chosen ESI-MS parameters (collision offset voltage, −25 V; temperature of the heated capillary, 200°C) seven different copper–solvent clusters with different m/z values could be detected in the mass range between m/z 60 and 160. So the concentration of the original species divides into several clusters, causing a decrease in the detection limit of this species. As a consequence, the signals of ion–solvent clusters in the analysis of real samples could be covered by signals of matrix compounds and so an identification in a complex matrix would be very difficult.

Brown et al. [30] examined the behaviour of many more different alkali, alkaline earth and transition metals by ESI-MS detection. Their results were the same as described above.

4.2.2. Metal ion-containing complexes

Organometallic complexes with weakly complexing ligands suffer from the same limitations as free metal ions, as can be seen for $TMSbCl_2$. The covalent part, the bondings to the three methyl groups will not be attacked by the ESI-MS process, while the weakly complexing chloride ligands will be partly exchanged. A distinction between species being formed as a consequence of gas-phase ligand replacement reactions and species being directly desorbed out of the bulk phase is not possible. In the presence of acetic acid, $TMSbCl_2$ hydrolyzes and solely forms the trimethylantimony acetic acid ester, because acetic acid is a stronger complexing ligand than chloride. When dissolved in water under neutral conditions $TMSbCl_2$ most probably forms the positively charged trimethylantimony hydroxide. The formation of a cation is also described for the homologue arsenic species, trimethylarsine oxide, at low pH (pK_a 3.6) [34].

If the stability constant of a complex is high, the ESI process will not change the structure of the complex and the detected species corresponds to the species actually existing in solution, as can be seen for ‘CuEDTA’ (Fig. 4). The ESI process provides the positively charged molecular ion $[CuEDTA]^+$. Anyway, ion–solvent clusters will be formed, par-

tially occurring in higher concentrations than the molecular ion itself. The charging state of copper is not changed.

The behaviour of different soluble Cu(I) and Cu(II) species by ESI-MS is described in the literature [35]. Cu(II)DMD [copper(II)–*N*,*N*′-disalicylidene-1,2-propylenediamine], a very stable copper complex, is always detected as protonated molecular ion $[CuDMDH]^+$. Different sample and sheath liquid compositions (2-propanol, hexane, acetic acid, acetonitrile) have no influence on the stability of the complex. Electrospraying of an aqueous solution of copper(II) acetate $Cu^{II}(CH_3COO)_2$ in the presence of acetonitrile (CH_3CN) instead, gave mostly (>95%) $[Cu+(CH_3CN)_2]^+$. Charge reduction of Cu(II) to Cu(I) takes place as well.

4.2.3. Covalent organometallic compounds

The ESI-MS spectra of the covalent organometallic selenocystamine SeCM and selenomethionine SeM are simple, as can be seen in Fig. 5 for SeCM. As a 'soft' ionization technique the energy of ESI-MS is too small to break up the covalent bonds between selenium atoms and the organic rests. Therefore, the species remain intact and will be detected as singly charged molecular ions. Ion–solvent clusters can be destroyed easily by increasing the temperature of the heated capillary or by application of CID, as described for selenomethionine. The spectrum, therefore, is dominated by the positively charged molecular ion and for this reason detection limits are much better than for free ions or weak complexes.

4.3. On-line CE–ESI-MS hyphenation for selenium speciation

The application of CE–ESI-MS for selenium speciation on an existing method using an alkaline background electrolyte gave unsatisfactory results. Analysis were badly reproducible and detection limits were rather high, because non-volatile buffers strongly affect the ESI process [12]: (i) high electrolyte concentrations may cause salt accumulation in the capillary vaporizer or in the ion source; (ii) the addition of ionic and neutral species in the sheath-flow competes for available charge in the ESI process, thus lowering the maximum sensitivity obtainable; (iii) the surface tension will be raised opposing the separation of droplets from the liquid front. The consequences are reduction of stable electrospray, loss of sensitivity and possibly a total electrical breakdown.

Using the volatile acidic electrolyte, the above-mentioned restrictions were no longer valid, and the detection limit for selenomethionine SeM could be improved by a factor of 36. All three Se species appeared solely as the protonated molecular ions. Since the CE separation method has not been optimized, the UV signal of the CE shows a relatively bad resolution for the three compounds, but they are baseline separated in the mass spectrometer due to extended capillary length. When running single standard applications, migration times were well reproducible and resolution of the compounds seemed adequate in mass spectrometric detection. Shifts of migration times occurred, when analyzing mixtures with increasing numbers of compounds, due to changes in sample conductivity and ion concentration. Migration times of analytes in a CE–ESI-MS separation can be affected by the sheath liquid composition as well [12]. Due to the potential gradient across the CE capillary, anions from both the sheath liquid and background electrolyte migrate towards the anode. When the anions differ in the two solutions, a moving ionic boundary is formed inside the capillary. Since the pH may be different within this moving boundary, the electroosmotic flow-rates, the effective charge on the analytes and their migration rates will change once they enter the boundary.

The separation of inorganic Se species gave unsatisfactory results. A possible reason may be the nearly identical values of electrolyte pH and lower p*K* values of these species; thus, Se (IV)/(VI) did not appear sufficiently cationic.

As can be seen, CE–ESI-MS is lacking in absolute concentration sensitivity. This is due, firstly, to the limited sample volume of CE that can be analyzed without compromising separation efficiency (<2% of the total capillary volume [36,37]) and, secondly, to the low efficiency of ion transport from the ESI source to the MS detector, with only approximately 10^{-5} of the ions formed in the ESI being detected [38]. To increase CE–MS detection limits, changes, methodical as well as technical, are necessary to

make the whole hyphenated system suitable for real sample analysis.

Future investigations will focus, firstly, on an improvement of the acidic CE separation of selenium species in order to make the two inorganic compounds Se(IV) and Se(VI) available as well. Secondly, the alkaline buffer system (Na_2CO_3–NaOH) of the CE separation method developed by Michalke and Schramel [27] will be adapted for better ESI-MS detection. On-line preconcentration steps (capillary isotachophoresis) in capillary electrophoresis will help to lower detection limits in order to make the system available for real samples, e.g. contaminated surface waters or body fluids.

References

[1] P. Quevauviller, Fresenius' J. Anal. Chem. 515 (1996) 345.
[2] S. Caroli (Ed.), Element Speciation in Bioorganic Chemistry, Wiley, New York, Chichester, 1996.
[3] J.V. Iribarne, P.J. Dziedzic, B.A. Thomson, 29th Annual Converence of Mass Spectrometry and Allied Topics, Minneapolis, MN, May 1981, p. 24.
[4] J.V. Iribarne, B.A. Thomson, J. Chem. Phys. 64 (1976) 2287.
[5] B.A. Thomson, J.V. Iribarne, J. Chem. Phys. 71 (1979) 2219.
[6] J.B. Fenn, J. Am. Soc. Mass Spectrom. 4 (1993) 524.
[7] A.P. Bruins, T.R. Covey, J.D. Henion, Anal. Chem. 59 (1987) 2642.
[8] A.P. Bruins, L.O.G. Weidolf, J.D. Henion, W.L. Budde, Anal. Chem. 59 (1987) 2647.
[9] M.A. Moseley, J.W. Jorgenson, J. Shabanowitz, D.F. Hunt, K.B. Tomer, J. Am. Soc. Mass Spectrom. 3 (1992) 289.
[10] F. Garcia, J.D. Henion, J. Chromatogr. 606 (1992) 237.
[11] D.C. Gale, R.D. Smith, Rapid Commun. Mass Spectrom. 7 (1993) 1017.
[12] Y. Takada, M. Sakairi, H. Koizumi, Anal. Chem. 67 (1995) 1474.
[13] J. Cai, J. Henion, J. Chromatogr. A 703 (1995) 667.
[14] A.J. Tomlinson, L.M. Benson, N.A. Guzman, S. Naylor, J. Chromatogr. A 744 (1996) 3.
[15] P.E. Jackson, P.R. Haddad, Trends Anal. Chem. 12 (1993) 231.
[16] M. Chen, R.M. Cassidy, J. Chromatogr. 640 (1993) 425.
[17] R. Guevremont, K.W.M. Siu, J.C.Y. Le Blanc, S.S. Berman, J. Am. Soc. Mass Spectrom. 3 (1992) 216.
[18] A.T. Blades, P. Jayaweera, M.G. Ikonomou, P. Kebarle, J. Chem. Phys. 92 (1990) 5900.
[19] X. Yu, M. Wojciechowski, C. Fenslau, Anal. Chem. 65 (1993) 1355.
[20] Y. Xu, X. Zhang, A.L. Yergey, J. Am. Soc. Mass Spectrom. 7 (1996) 25.
[21] T.G. Huggins, J.D. Henion, Electrophoresis 14 (1993) 531.
[22] J.J. Corr, J.F. Anacleto, Anal. Chem. 68 (1996) 2155.
[23] B. Michalke, P. Schramel, Fresenius' J. Anal. Chem. 257 (1997) 594.
[24] M. Albert, C. Demesmay, J.L. Rocca, Fresenius' J. Anal. Chem. 351 (1995) 426.
[25] M. Dood, S.L. Pergantis, W.R. Cullen, Appl. Organomet. Chem. 6 (1992) 207.
[26] G.T. Morgan, V.E. Yarsley, Proc. R. Soc. 110 (1926) 534.
[27] B. Michalke, P. Schramel, Electrophoresis 19 (1998) 270.
[28] R.E. Smith, A.E. Martell, Critical Stability Constants, Plenum, New York, 1989.
[29] J. Lintschinger, O. Schramel, A. Kettrup, Fresenius' J. Anal. Chem. (1998) in press.
[30] F.B. Brown, L.K. Olson, J.A. Caruso, J. Anal. Atom. Spectrom. 11 (1996) 633.
[31] A.P. Bruins, Trends Anal. Chem. 13 (1994) 37.
[32] G.R. Agnes, G. Horlick, Appl. Spectrosc. 48 (1994) 655.
[33] T. Blades, P. Jayaweera, M.G. Ikonomou, P. Kebarle, Int. J. Mass Spectrom. Ion Processes 102 (1990) 251.
[34] B.D. Chernokal'skii, L.A. Vorob'eva, Zh. Obsch. Khim. 43 (1973) 1939.
[35] C.L. Gatlin, F. Turecek, T. Valsar, Anal. Chem. 66 (1994) 3950.
[36] J. Cai, Z. El Rassi, J. Liq. Chromatogr. 15 (1992) 1179.
[37] J. Cai, Z. El Rassi, J. Liq. Chromatogr. 16 (1993) 2007.
[38] R.D. Smith, J.A. Loo, C.G. Edmonds, C.J. Barinaga, H.R. Udseth, J. Chromatogr. 516 (1990) 157.