Journal of Chromatography A, 652 (1993) 547–553
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 304

Determination of organolead and organoselenium compounds by micellar electrokinetic chromatography

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ABSTRACT

The separation of selected organolead and organoselenium compounds by high-performance capillary electrophoresis was investigated. Satisfactory separation was achieved using β-cyclodextrin-modified micellar electrokinetic chromatography with on-column UV detection at 210 nm. This method was applied to the analysis of trialkyllead chlorides and organoselenium compounds in spiked distilled water and environmental samples. In addition, the effect of pH, sodium dodecyl sulphate and β-cyclodextrin concentrations on the migration behaviour of these compounds was examined.

INTRODUCTION

There has been a tremendous growth in the use of capillary electrophoresis (CE) in recent years [1–10]. The popularity of the technique can be partly attributed to its potential to achieve high efficiencies and its ease of operation. Many applications have been developed for biomedical [1–4] and pharmaceutical analysis [5–7]. However, only a few papers on the analysis of environmental pollutants using CE have appeared to date [8–10]. In view of the need for low detection limits, small sample volumes and rapid monitoring in environmental trace analysis, CE is a viable alternative for such applications.

Organic compounds of heavy metals such as lead and selenium have been shown to be carcinogenic [11]. These compounds are disseminated into the environment via mining, refining, paints and, in the case of tetraalkylleads, as antiknock additives in gasoline. Tetraalkyllead compounds undergo photochemical and metabolic dealkylation to form the trialkyllead compounds, which are chemically active and thermally sensitive. The most common technique used for the detection and determination of these trace elements is gas chromatography (GC) [12,13] with atomic absorption spectrometry because of their high element specificity and sensitivity. A major disadvantage of this technique is the need for highly sophisticated detectors (graphite furnace atomic absorption or microwave-excited helium plasma detector). Further purification steps are also needed to remove interferences from other forms of organic and inorganic compounds. More recently, high-performance liquid chromatography (HPLC) has been used for the determination of these compounds. For the determination of trialkyllead compounds, HPLC with on-line extraction does not yield better sensitivity than GC [14].

Since the first description of micellar electrokinetic chromatography (MEKC) by Terabe *et al.* [15], a large number of applications based on this technique have been reported [16–19]. In MEKC, a buffer solution containing a surfactant [*e.g.*, sodium dodecyl sulphate (SDS)] is used as the electrophoretic medium. The main advantage of MEKC is that both neutral and charged compounds can be separated by it. Further, by

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0021-9673/93/$06.00

introducing suitable additives (*e.g.*, cyclodextrins and complexing agents) into the buffer, unique separation mechanisms can be exploited [20]. In this work, the separation of a mixture of two organolead and two organoselenium compounds using MEKC with β-cyclodextrin was examined. The method was applied to the determination of these compounds in spiked distilled water and environmental samples. In addition, the effects of pH and SDS and β-cyclodextrin concentrations on the migration behaviour of these compounds were investigated.

EXPERIMENTAL

Experiments were conducted on a laboratory-built CE system. A Spellman (Plainview, NY, USA) Model RM15P10KD power supply, which is capable of delivering up to 15 kV, was used. Fused-silica capillary tubing of 44 cm × 50 μm I.D. was obtained from Polymicro Technologies (Phoenix, AZ, USA). An ISCO (Lincoln, NE, USA) CV^4 variable-wavelength UV detector with the wavelength set at 210 nm was used for the detection of peaks. Chromatographic data were collected and analysed using a Hewlett-Packard (Avondale, PA, USA) Model HP3394A integrator.

The pH of the buffer solutions used in the CE system was adjusted by mixing 25 m*M* sodium tetraborate and 25 m*M* sodium dihydrogenphosphate solutions. β-Cyclodextrin and sodium dodecyl sulphate (SDS) were purchased from Fluka (Buchs, Switzerland). Trimethyllead chloride (TML), triethyllead chloride (TEL), diphenyl selenide (Ph_2Se) and phenyl selenyl chloride (PhSeCl) were obtained from Johnson Matthey (Ward Hill, MA, USA). Standard solutions in methanol having a concentration of 666 ppm for TML, 400 ppm for TEL, 466 ppm for diphenyl selenide and 600 ppm for phenylselenyl chloride were used. Sample solutions were injected hydrodynamically at a height of 2 cm and over an injection time of 4 s. Each injection was calculated [21] to be *ca.* 0.3 nl.

Extraction from spiked distilled water

Trimethyllead and triethyllead chloride. A known amount of TML and TEL was added to 500 ml of distilled water and shaken for 5 min. The sample was evaporated under vacuum at 55°C until a residual volume of about 10 ml remained. The sample was quantitatively transferred into a 100-ml separating funnel and the volume was adjusted to 25 ml with distilled water. About 8–9 g of sodium chloride were added and the pH was brought to below 10, if necessary. The sample was then extracted twice with 25-ml portions of chloroform. The extracts were combined and dried with magnesium sulphate. The chloroform extract was filtered and evaporated to dryness under vacuum at 40°C. The TEL and TML thus obtained were dissolved in methanol and the solution was analysed by CE.

Diphenyl selenide and phenyl selenyl chloride. A known amount of the organoselenium compounds was dissolved in 30 ml of methanol and was then added to distilled water, giving a total volume of 200 ml in a 250-ml separating funnel. The sample was extracted three times with 30 ml of chloroform. The extracts were combined, dried with magnesium sulphate, filtered and evaporated under vacuum at 40°C until a residual volume of 2 ml of extract was left. The sample was then air dried at room temperature (25°C), after which the sample was dissolved in methanol and introduced into the CE system for analysis.

Analysis of environmental samples

Water samples (1.5 l) were collected from drains next to a car park. The samples were filtered and then extracted using procedures similar to those for TML and TEL.

RESULTS AND DISCUSSION

As the organometallic compounds are heat and light sensitive, the ionic strength of the electrophoretic medium (the concentrations of the phosphate and borate buffer solutions) used was kept low, *i.e.*, at 25 m*M* each for the phosphate and borate buffer solutions. This was to prevent excessive joule heating, which is likely to occur when high ionic strength buffer solutions are used. Thus, degradation of compounds

inside the capillary during the run was prevented or at least minimized.

Preliminary experiments were conducted to separate the organolead and selenium compounds using CZE conditions, *i.e.*, at pH 6.0, 7.0 and 8.0. The results obtained for the migration times at different pH values are shown in Fig. 1. It can be seen that with an increase in pH, there is an increase in the electroosmotic flow velocity. This is indicated by the decrease in migration times of the neutral diphenyl selenide, which co-eluted with the solvent, the unretained solute (methanol). This could be due to the increase in ionization of the surface functional groups with pH at the inside of the wall, which subsequently resulted in an increase in the zeta potential. The effect of the increased electroosmotic flow velocity could be seen from the decrease in migration times for neutral organoselenium compounds, Ph_2Se and PhSeCl. On the other hand, an opposite trend was observed for trimethyllead chloride (TML) while the migration times for triethyllead chloride (TEL) were fairly constant with changes in pH.

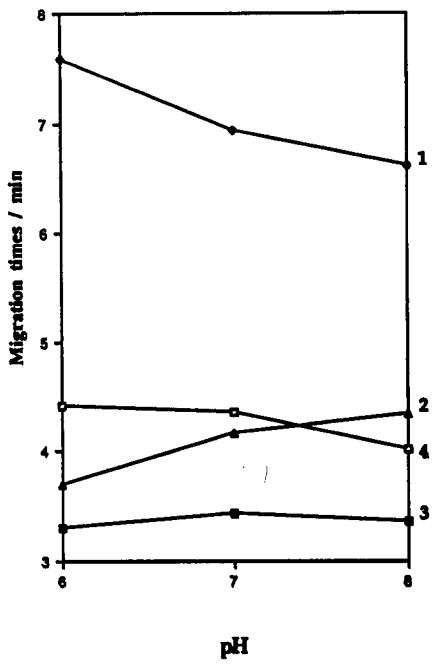

Fig. 1. Variation of migration time with pH. 1 = Phenyl selenyl chloride; 2 = trimethyllead chloride; 3 = triethyllead chloride; 4 = diphenyl selenide.

TEL and TML are known to be partially dissociated [22] in aqueous medium, forming PbR_3^+, as shown in the equation

$$PbR_3Cl \overset{k}{\rightleftharpoons} PbR_3^+ + Cl^-$$

where k = dissociation constant. Thus, these compounds carry a partial positive charge under these conditions. Consequently, they are attracted to the cathode, with the result that their migration times are shorter than that of the solvent (shown by the plot for Ph_2Se) at pH 6 and 7 in Fig. 1. With an increase in pH, there was an increase in the migration time for TML, and at pH 8 the TML peak was observed to elute after the solvent peak (compare the lines at pH 8 for Ph_2Se and TML). The increase in migration time of TML with pH (*i.e.*, a decrease in the apparent velocity) in spite of an increase in electroosmotic flow velocity could be attributed to an increase in the borate concentration in the electrophoretic medium at higher pH. The borate ions would tend to envelop the positively charged TML, resulting in partial neutralization of the positive charge on TML. Subsequently, the electrophoretic velocity towards the cathode was reduced. At high pH (pH > 8), TML was completely neutralized by the borate ions, and thus eluted after the solvent. The borate ions, on the other hand, had no significant effect on the migration times of TEL. This could be due to steric hindrance caused by the three bulky ethyl substituent groups attached to lead. Subsequently, the borate ions were prevented from approaching the central lead atom. Hence the partially positive charged TEL was the first to be eluted and it always migrated before the solvent peak at the pH values investigated.

Another interesting observation is the migration order of TML and TEL. It was noted that TEL always migrated earlier than TML under CZE conditions. This was surprising as TML, being smaller and thus possessing a higher charge-to-radius ratio, would be expected to have a higher electrophoretic velocity than TEL towards the cathode. Hence it would be expected to migrate faster than TEL. However, it is conceivable that a higher degree of ion pairing exists between borate and TML (because of the

smaller size compared with that of TEL), which reduces the effective charge on the TML. Consequently, the latter possesses a lower net velocity towards the cathode.

For organoselenium compounds, no unusual trends were observed. As they are neutral or dissociate only slightly, they either co-migrated with the solvent (*e.g.*, Ph_2Se) or migrated after the solvent peak (*e.g.*, PhSeCl).

In order to improve the separation of the neutral compounds, MEKC was performed. The effect of SDS concentration on the separation of this group of compounds is illustrated in Fig. 2. SDS concentrations of 25 and 50 m*M* were considered. The migration order followed the expected trend in MEKC, *i.e.*, the more hydrophobic solutes were retained longer than the less hydrophobic species. Hence, in this instance, TEL migrated after TML, followed by Ph_2Se and PhSeCl.

Although separation of peaks could be achieved for the four solutes in MEKC, peak tailing was observed for TEL and PhSeCl. In view of this problem, β-CD was added to the electrophoretic medium to improve the peak shape. The results obtained for different β-CD concentrations on the separation are illustrated in Fig. 3. It can be seen that the migration times of all the solutes decreased with increase in β-CD concentration. This could be due to the competitive interaction between the solutes with β-CD and SDS. As β-CD is neutral, it would migrate faster than SDS towards the cathode. Hence solutes forming host–guest complexes with β-CD would be brought to the cathode at a faster rate. It is noteworthy that at 15 m*M* β-CD, crossover of peaks for TEL and Ph_2Se is observed. At lower β-CD concentration, TEL migrated faster than Ph_2Se. At higher β-CD concentrations, because interaction with β-CD depends on the compatibility of the molecular

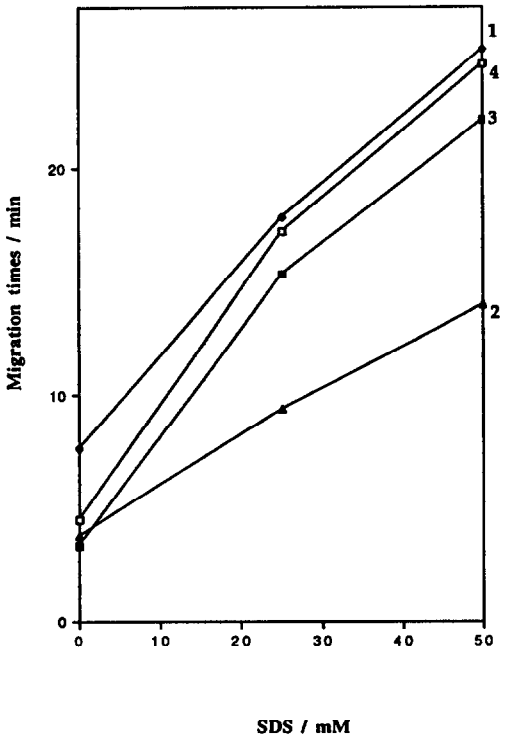

Fig. 2. Variation of migration time with SDS concentration. Compounds as in Fig. 1. Electrophoretic conditions: Micellar solution in 25 m*M* phosphate–borate buffer (pH 6.0); applied voltage, 15 kV.

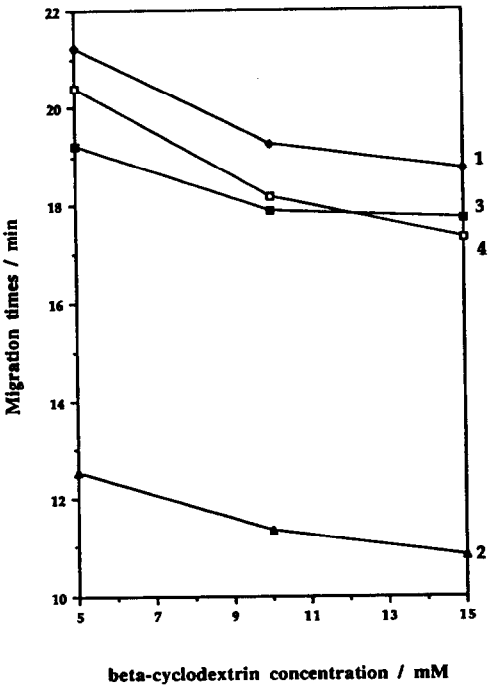

Fig. 3. Variation of migration time with β-CD concentration. Compounds as in Fig. 1. Electrophoretic conditions: 50 m*M* SDS in 25 m*M* phosphate–borate buffer (pH 6.0); applied voltage, 15 kV.

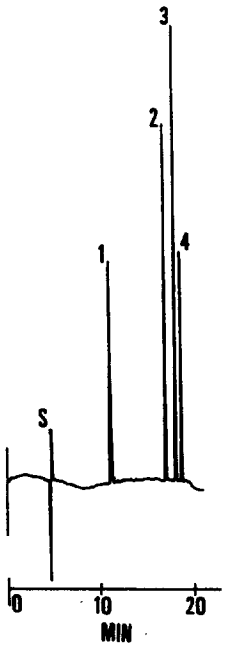

Fig. 4. Typical electropherogram obtained for organolead and organoselenium compounds. Peaks: S = solvent (methanol); 1 = trimethyllead chloride; 2 = triethyllead chloride; 3 = diphenyl selenide; 4 = phenylselenyl chloride. Electrophoretic conditions: 50 m*M* SDS and 5 m*M* β-cyclodextrin in 25 m*M* phosphate–borate buffer (pH 6.0); separation tube, 44 cm × 50 μm I.D. fused silica; applied voltage, 15 kV; wavelength, 210 nm.

size of the solutes with the cavity of β-CD and Ph_2Se tends to be incorporated more readily into the cavity of β-CD, it migrated earlier than TEL. It was also observed that at high β-CD concentrations, the resolution between TEL and Ph_2Se was unsatisfactory, and irreproducible migration times were obtained after every injection unless flushing of the column with 0.1 *M* NaOH was carried out for at least 15 min between runs. This problem was not observed at lower β-CD concentrations. Of the three concentrations of β-CD investigated, it was found that 5 m*M* β-CD offered sharp peaks and the best resolution. Hence the conditions of pH 6.0, 50 m*M* SDS and 5 m*M* β-CD were used for subsequent investigations. A typical electropherogram for this group of compounds is shown in Fig. 4. Typically, plate numbers greater than 200 000 were obtained for the separations.

Analysis of spiked distilled water

Linear calibration graphs in the range 80–600 ppm were obtained for the four solutes. The detection limits (signal-to-noise ratio = 3), correlation coefficients and recoveries are given in Table I. Typical electropherograms of the extracted samples for the two groups of compounds are illustrated in Fig. 5a and b. Water samples (1.5 l) from drains at a heavily used car park were collected for analysis. The organic compounds under investigation were not detected. The reason could either be that the compounds were present at levels below the detection limits, or they could have undergone photochemical degradation [13]. A typical electropherogram for the analysis of the water samples is shown in Fig. 6a. To confirm that the absence of these compounds was not attributable to the analytical procedure, known amounts of TML and TEL were added to 500 ml of the water sample before

TABLE I

ABSOLUTE DETECTION LIMITS, CORRELATION COEFFICIENTS AND RECOVERIES OF ORGANOLEAD AND ORGANOSELENIUM COMPOUNDS

Compound	Detection limit (pg)	Correlation coefficient	Recovery (%)
Trimethyllead chloride	20	0.998	90
Triethyllead chloride	8	0.999	80
Diphenyl selenide	9	0.998	104
Phenylselenyl chloride	18	0.999	83

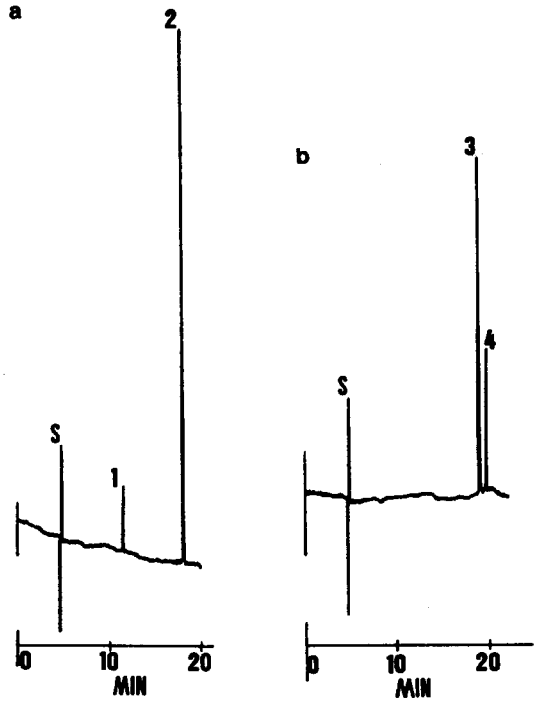

Fig. 5. Electropherograms obtained after extraction of (a) TML and TEL from spiked distilled water and (b) Ph_2Se and PhSeCl from spiked distilled water. Peaks as in Fig. 4. Electrophoretic conditions: 50 m*M* SDS and 5 m*M* β-cyclodextrin in 25 m*M* phosphate–borate buffer (pH 6.0); separation tube, 44 cm × 50 μm I.D. fused silica; applied voltage, 15 kV; wavelength, 210 nm.

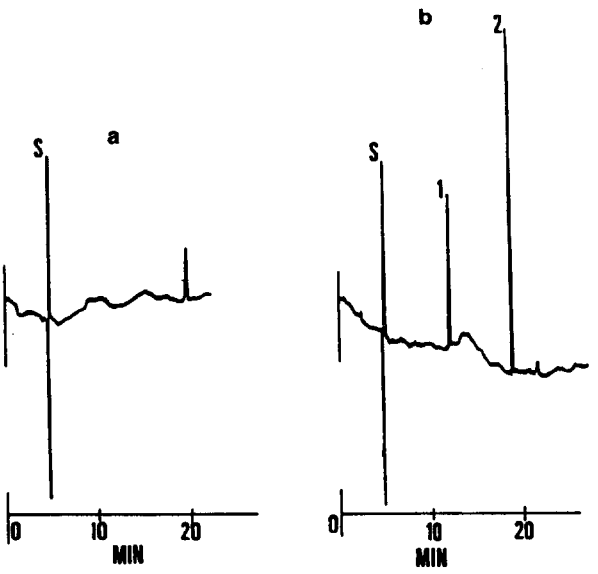

Fig. 6. Electropherograms obtained for (a) a water sample collected at a heavily used car park and (b) a water sample spiked with TML and TEL. Peak identification as in Fig. 4.

extraction. Both components were detected at the expected migration times. A typical electropherogram for the spiked water sample is shown in Fig. 6b.

CONCLUSIONS

The feasibility of using MEKC for the separation of a mixture of organolead and organoselenium compounds has been demonstrated. By the addition of β-cyclodextrin to the electrophoretic medium, improvements in the peak shapes were observed. To the best of our knowledge, this is the first reported analysis of these groups of compounds by MEKC. High separation efficiencies and detection limits comparable to those obtained by GC were achieved. However, the primary advantage of MEKC over GC is that the former is performed at temperatures that prevent potential thermal degradation of the compounds of interest. Given this advantage, the method may be used in analyses for other environmental pollutants with little modification of the fundamental conditions used in this work. Further, the technique offers rapid analyses, and low running costs and is aqueous rather than organic solvent based, all these being advantageous over traditional chromatographic procedures. An additional advantage is the potential incorporation into the MEKC method of enrichment procedures currently being exploited in CE such as isotachophoresis [23–25], field amplification injection [26–28] and in-capillary solid-phase extraction techniques [29,30]. None of these are available to GC for improving its detection limits.

ACKNOWLEDGEMENT

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

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