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Short communication

Investigation of the separation efficiency of hydrophobic compounds in suspension electrokinetic chromatography

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Abstract

A suspension of reversed-phase particles is used as a pseudo-stationary phase in capillary electrokinetic chromatography. For these apolar particles, a dynamic coating with ionic detergents is needed to form stable suspensions and to introduce the charges responsible for their mobility in the electric field. This is achieved by the addition of surfactants to the electrolyte. The impact of the particles on peak broadening is shown for the separation of polycyclic aromatic hydrocarbons, which were used as test solutes.

Keywords: Separation efficiency; Suspension electrokinetic chromatography; Polynuclear aromatic compounds

1. Introduction

Since the introduction of micelles as a pseudo-stationary phase by Terabe et al. [1], electrokinetic chromatography (EKC) has become a famous method for the separation of uncharged molecules and enantiomers [2–4]. Besides the micelles, macrocyclic compounds like cyclodextrins [5] and resorcarenes [6] as well as polymeric phases like oligomeric sodium-10-undecylenate [7] and starburst dendrimers [8] have been employed.

Another approach to achieve selectivity in EKC is the use of a suspension of chromatographic particles (SEKC) as a pseudo-stationary phase. With this system, adaptation of selectivity known from chromatography is possible to electrophoresis. Therefore, separation problems should be easier to solve.

In our first study in the field of SEKC, we separated nine phenol derivatives using reversed-phase particles [9]. Because of baseline disturbance of the particles in UV detection, a discontinuous set-up was developed. Due to the polar character of the phenols, they had very small capacity factors. Therefore, the influence of the particles was small and band broadening due to the particles could not be determined precisely.

In this study, we wanted to investigate the impact of particles on separation. Some polycyclic aromatic hydrocarbons (PAHs) were chosen as test solutes due to their high capacity factors. Continuous SEKC was possible due to the fluorescence activity of the analytes.

2. Experimental

2.1. Chemicals

All polycyclic aromatic hydrocarbons (PAHs) were purchased from Supelco (Deisenhofen, Ger-

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many) and SDS was from Merck (Darmstadt, Germany). Urea and β-cyclodextrin (β-CD) were used as received from Fluka (Neu-Ulm, Germany).

Buffer solutions were made from reagent-grade chemicals (Merck): sodium tetraborate and sodium phosphate, adjusted to the desired pH with NaOH.

Non-porous RP-18 particles, with a size of 500 nm (Monospher R), were provided by Merck.

2.2. Apparatus

The electrophoresis equipment consisted of a laboratory-built CE box, a high-voltage power supply (F.u.G. Elektronik, Rosenheim, Germany), a variable UV detection system from Dionex (Idstein, Germany) and a Spectroflow 980 fluorescence detector (Applied Biosystems, Weiterstadt, Germany), which was modified for on-column detection [10]. The excitation wavelength was 245 nm and the fluorescence was separated from the scattered light with a 280-nm cut-off filter. Untreated fused-silica capillary tubes with an inner diameter (I.D.) of 75 μm were purchased from Chromatographieservice (Langerwehe, Germany).

2.3. Preparation of suspensions

Preparation of the suspensions was performed in two steps: (1) suspension of the particles in the buffer solution containing SDS using ultrasound and (2) addition of modifier (urea and β-CD). To achieve maximum reliability, the suspension was treated in an ultrasonic bath for several minutes before each injection.

3. Theory

3.1. Suspension of chromatographic particles

The use of chromatographic particles as a pseudo-stationary phase can be regarded as a combination of electrophoresis and chromatography. Separation is achieved according to different distribution coefficients between the buffer and the surface of the particle.

In EKC, the resolution for uncharged species depends on the velocity of the electroosmotic flow (EOF) and that of the pseudo-stationary phase, as described by Terabe et al. [2]. The greatest influence on retention time will be found for particles with an opposite migration direction to that of the analyte. This effect increases with increasing velocity differences between the analyte and the particle. Charged particles, based on classical stationary phases known from pressure-driven chromatography (normal- (NP) and reversed-phase (RP) particles are mostly used), with sufficient mobility can be formed depending on the pH (NP), using surfactants that interact with the apolar surface (RP-18), or by synthesizing particles that have a certain degree of charged groups.

3.2. Peak broadening compared to other electrokinetic techniques

The dependence of efficiency on the strength of the electric field (H/E) for MEKC is discussed in detail by Terabe et al. [3] and by Sepaniak and Cole [11]. Total band broadening (H_{tot}) is described as the sum of five independent parameters.

$$H_{tot} = H_l + H_{m\,(m)} + H_{aq\,(m)} + H_T + H_{ep\,(m)} \quad (1)$$

where H_l is longitudinal diffusion, $H_{m\,(m)}$ is adsorption/ desorption kinetics, $H_{aq\,(m)}$ is inter-micelle mass transfer, H_T is radial temperature gradient, and $H_{ep\,(m)}$ is dispersion due to different mobilities of the micelles.

Among these five factors, H_l, H_m and $H_{ep\,(m)}$ are found to contribute significantly to band broadening in MEKC. The analogous discussion can also be applied to SEKC where the total peak broadening is composed of

$$H_{tot} = H_l + H_{m\,(p)} + H_{aq\,(p)} + H_T + H_{ep\,(p)} \quad (2)$$

where $H_{aq\,(p)}$ is inter-particle mass transfer, and $H_{ep\,(p)}$ is dispersion due to different particle velocities.

For a mixed system (MEKC and SEKC due to the need to suspend the particles), one gets Eq. (3):

$$H_{tot} = H_l + H_{m\,(m)} + H_{aq\,(m)} + H_{ep\,(m)} + H_T + H_{m\,(p)} + H_{aq\,(p)} + H_{ep\,(p)} \quad (3)$$

In cases where micelles have negligible influence, $H_{m\,(m)}$, $H_{aq\,(m)}$ and $H_{ep\,(m)}$ will not be important, leading to Eq. (4):

$$H_{tot} = H_l + H_{m\,(p)} + H_T + H_{aq\,(p)} + H_{ep\,(p)} \quad (4)$$

4. Results and discussion

4.1. Buffer composition

As RP-particles are not moistened by water, the addition of surfactants, such as SDS, at concentrations at least at the critical micellar concentration (cmc) to the buffer solution and to the suspension is necessary to form a stable suspension. SDS covers the surface of the particles, forming a charged outer sphere. The characteristics of these particle suspensions are described in detail elsewhere [9]. As the solubility of PAHs is very low in aqueous buffers, additives are necessary for this system. The most commonly used additive is urea [12], which destroys the hydrogen bond structure of water. Another possibility to increase the solubility is the addition of cyclodextrins, which form inclusion complexes with analytes of suitable size [13]. In this study, we used β-CD, which increases the solubility of the smaller PAHs, such as naphthalene, phenanthrene, anthracene and 2,3-benzofluorene, which were employed as test solutes.

The addition of modifiers used in HPLC, such as methanol or acetonitrile, is not possible, due to their influence on the particle coating [14]. As the modifier molecules replace the surfactant molecules, the dynamic coating is destroyed and the particles agglomerate, causing the suspension to become unstable. This is obviously a disadvantage of these particles, however, preliminary investigations are possible, nevertheless.

Due to the high viscosity of the buffer, sedimentation does not occur for at least 1 h, so the stability of the particle suspension throughout the separation is good.

4.2. Separation of PAHs

In the first step, the influence of the electrolyte and the buffer additives on the separation was investigated. The electropherogram is shown in Fig. 1, where no separation was found. This is due to the very small amount of SDS in the buffer. Additionally, the uncharged β-CD itself cannot lead to any separation. Therefore, all PAHs migrate with the velocity of the EOF. Separation of the PAHs in this system is achieved when a suspension of particles is applied. The separation and identification of the compounds are shown in Fig. 1.

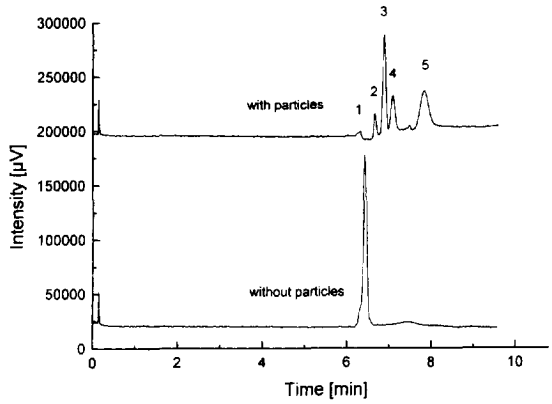

Fig. 1. Separation of PAHs with particles. Conditions: buffer, 10 m*M* sodium tetraborate, 5 m*M* sodium phosphate, 4 m*M* sodium dodecylsulfate, 4 *M* urea, 40 m*M* β-CD, pH 9.5. Suspension: 3% (w/v). Injection: electrokinetic for 10 s, 10 kV. Separation: 30 kV. Capillary: 1 m total length, 0.7 m effective length. Detection: fluorescence, excitation, 245 nm; emission, 280 nm cut-off. Peakas: 1 = EOF; 2 = naphthalene (12.5 ppm); 3 = anthracene; 4 = phenanthrene; 5 = 2,3-benzofluorene (5 ppm each).

In reversed-phase HPLC, the retention order of the PAHs corresponds to increasing hydrophobicity. The retention order is found to be naphthalene, phenanthrene, anthracene and 2,3-benzofluorene. As the retention in SEKC is dependent on the distribution of the analyte between the buffer containing β-CD and the particles, the order is not exactly the same as in HPLC. For phenanthrene and anthracene, the order is reversed. This is presumably due to the stronger inclusion of the linear anthracene in β-CD and therefore decreased interaction with the particles.

In order to quantify peak broadening due to the particles and to compare these results to MEKC, we measured the *H/E* dependency of the compounds. The resulting data are shown in Fig. 2. The shapes of these curves were as expected from theory. For small voltages, the increase in plate height is attributed to longitudinal diffusion, whereas the increase found for larger voltages results from insufficient heat dissipation in the particle zone and therefore in-

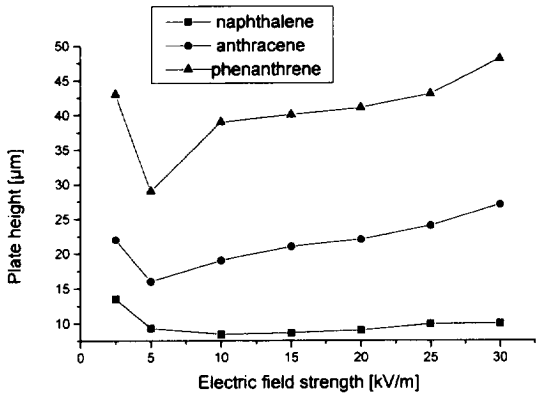

Fig. 2. Dependence of plate height on electric field strength. Conditions: buffer, 10 m*M* sodium tetraborate, 5 m*M* sodium phosphate, 4 m*M* sodium dodecylsulfate, 4 *M* urea, 20 m*M* β-CD, pH 9.5. Suspension: 3% (w/v). Injection: electrokinetic for 10 s, 10 kV. Separation: 30 kV. Capillary: 1 m total length, 0.7 m effective length. Detection: fluorescence, excitation, 245 nm; emission, 280 nm cut-off.

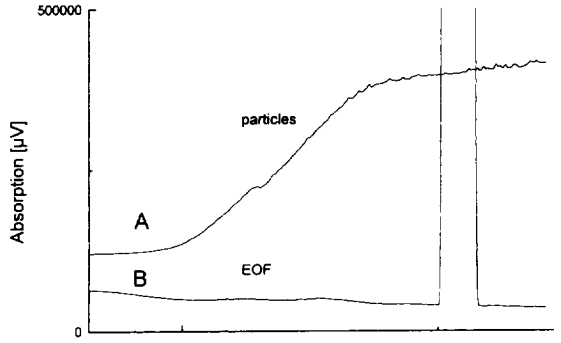

Fig. 3. Zone broadening due to different particle velocities compared to the EOF signal. Conditions: buffer, 10 m*M* sodium tetraborate, 5 m*M* sodium phosphate, 4 m*M* sodium dodecylsulfate, 4 *M* urea, 40 m*M* β-CD. Detection: UV at 220 nm. (A) Particle zone. Suspension: 3% (w/v). Injection: as plug from cathodic end, −23.4 kV. Capillary: 78 cm total length, 16 cm effective length. (B) EOF zone. Injection: hydrostatic, 30 s, 10 cm. Separation: 23.4 kV. Capillary: 78 cm total length, 62 cm effective length. Separation: 30 kV.

creased diffusion coefficients [15]. This assumption is supported by the shape of the Ohm plot, which shows significant deviation from linearity at field strengths over 15 kV/m.

The increase in plate height for the larger PAHs is attributed to different mobilities of the particles. The influence of $H_{ep\ (p)}$ on band broadening increases for higher capacity factors. Even in MEKC, the most important peak broadening was attributed to the microheterogeneity of the micelles, which resulted in different micelle velocities [2]. One possible proof for the occurrence of different velocities is the curve shape found for a plug of particles injected electrokinetically. Fig. 3 (curve A) shows the smoothly increasing baseline of the particle plug in comparison to the sharp EOF signal (curve B). This result is in good agreement with a study on the separation of inorganic fine particles made by Quang et al. [16]. They found that particles' signals were broader than those of "point charge" molecules, which was attributed to the heterogeneity of the ζ-potentials of the particles [17].

In the case where particles have equal velocities, better efficiencies are expected for substances with high capacity factors. They should at least be in the range of those found for naphthalene, which is around 100 000/m.

4.3. Influence of modifier

As separation is due to the different distribution of the analytes between the particles and the buffer, variation in the β-CD concentration is expected to have a major impact. Fig. 4 shows separations with different concentrations of β-CD ranging from 10 to 60 m*M*. The decrease in EOF is a consequence of increasing viscosity with higher amounts of β-CD. Increasing amounts of β-CD result in shorter retention times of the analysed PAHs due to decreased

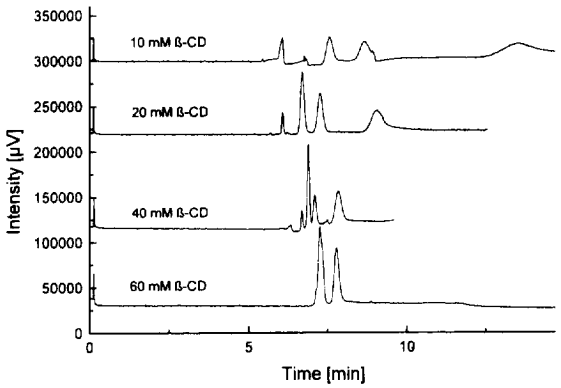

Fig. 4. Influence of the concentration of β-CD on the separation of PAHs. Conditions as described in Fig. 1.

Table 1
Capacity factors for different concentrations of β-CD

	Naphthalene	Anthracene	Phenanthrene	2,3-Benzofluorene	Particle migration time (min)
10 m*M* β-CD	0.33	1.64	4.2	21	10.3
20 m*M* β-CD	0.12	0.41	0.73	2.8	11.5
40 m*M* β-CD	0.11	0.22	0.3	0.69	12.1
60 m*M* β-CD	0.1	0.1	0.1	0.3	13.3

inclusion. At a concentration of 60 m*M* β-CD, no separation was achieved.

Capacity factors are calculated as described by Terabe et al. [2]. They vary from 0.1 to 21, depending on the concentration of β-CD (see Table 1). The particle velocities are determined as described in Ref. [9] and are given in Table 1.

Peak broadening is found to increase at lower concentrations of β-CD. This could be due partly to insufficient solubility of the PAHs in the buffer system, but it is most probably a consequence of the different particle velocities as stated above.

5. Concluding remarks

This preliminary study towards the development of new particles shows the impact of particles on separation efficiency. Highly hydrophobic compounds, such as PAHs, were chosen as test solutes. The order of retention is found to be similar to HPLC. The exchange of phenanthrene and anthracene is attributed to the use of β-CD as a modifier and its ability to form inclusion complexes with the PAHs. Efficiencies were found to be in the range of 20 000/m to 100 000/m. The decrease in plate numbers for analytes with high capacity factors is attributed to the differences in velocity of the particles.

Unfortunately, the lack of stability of the particles used could not be overcome. Therefore, there is a great need to develop particles that are optimized for SEKC. These particles should have a charged surface and functional groups for interaction that are chemically bonded to the particle support material. Further investigations are planned on the production and application of these particles.

Acknowledgments

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