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Capillary electrokinetic chromatography with a suspension of chromatographic particles

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Abstract

The use of a suspension of chromatographic particles as a pseudo-stationary phase in capillary electrokinetic chromatography is demonstrated. The separation of nine phenol derivatives is presented to demonstrate the influence of the particles. Reversed-phase particles with a diameter of 1.5 μm were chosen. These particles were coated with a surfactant to form a stable suspension. The capacity factor of the chromatographic separation can be varied by changing the particle concentration, independent of other parameters. To avoid light scattering at the particles, a discontinuous set-up was developed.

1. Introduction

As it is not possible to separate uncharged analytes or analytes with equal mobilities in capillary zone electrophoresis (CZE), new analytical techniques such as micellar electrokinetic chromatography (MEKC) [1–3], cyclodextrin-modified electrokinetic chromatography (CD-EKC) [4] and microemulsion electrokinetic chromatography (MEEKC) [5,6] have been developed. In MEKC and MEEKC it is not possible to increase the amount of the organic modifier beyond a certain limit, otherwise the stability of the microemulsion can no longer be guaranteed and, in the case of micelles as a pseudo-stationary phase, inverse micelles are formed. A possible solution is the use of a suspension of chromatographic particles.

Although the plate numbers in CZE are very high owing to the plug profile (up to 1 000 000), the selectivity may be the limiting factor. It can be improved, however, by the addition of a pseudo-stationary phase. Velocity differences of the analytes are enlarged according to the distribution between a pseudo-stationary phase and the buffer solution.

Chromatographic and especially reversed-phase (RP) particles show different selectivities (known in HPLC) compared with MEKC and MEEKC, so separation problems may be solved more easily. The selectivity of chromatographic particles is high, so the versatility of the EKC systems is increased by the incorporation of chromatographic particles as a pseudo-stationary phase. The capacity factor in the new method is influenced by the content of organic modifier and by the concentration of the particles. Particles with charged functional groups form stable suspensions in aqueous buffers. Particles with apolar surfaces have to be coated dynamically to

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form a stable suspension. In this study, we adapted a well known CZE separation of nine selected phenols to discontinuous SEKC. As in chromatography the separation of phenols is made with RP particles, we chose RP-18 particles with a diameter of 1.5 μm. Dynamic coating was achieved by the addition of sodium dodecyl sulfate.

2. Experimental

2.1. Chemicals

All phenols were purchased from Supelco (Bad Homburg, Germany) and sodium dodecylsulfate (SDS) from Merck (Darmstadt, Germany). Toluene and Sudan III were used as received from Fluka (Neu-Ulm, Germany).

Buffer solutions were prepared from analytical-reagent grade chemicals (Merck) used for electrophoresis: sodium tetraborate and sodium phosphate, pH adjusted with NaOH. Samples for hydrodynamic injection were prepared in water.

Non-porous Chromspher UOP RP-18 particles with a size of 1.5 μm were provided by Chrompack (Frankfurt, Germany).

2.2. Preparation of suspensions (dynamic particle coating)

The particle suspensions are manufactured ultrasonically. SDS is added to the buffer as a surfactant to achieve a charged surface. This suspension remains stable for more than 1 h. Subsequently, significant sedimentation of the particles is observed, leading to a decrease in particle concentration and to a lower reproducibility of retention times. To achieve maximum reliability, the suspension is treated in an ultrasonic bath for several minutes before each injection.

Particle concentrations of more than 10% (w/v) often result in a blocked capillary, so this concentration seems to be the limit for easy handling. It is possible to regenerate blocked capillaries by using an ultrasonic bath or pressure.

2.3. Instrumentation

The modular electrophoresis instrument used consisted of a Lambda 1000 variable-wavelength UV detection system (Bischoff, Leonberg, Germany), an HCN 6M-30000 high-voltage power supply (FUG, Rosenheim, Germany) and a PRINCE basic electrophoresis apparatus with autosampler (Lauer Labs., Netherlands). Fused-silica capillary tubes with an I.D. of 75 μm were purchased from Chromatographieservice (Düsseldorf, Germany). Detection was achieved on-column.

2.4. Separation conditions

For the separation of phenols, different amounts (0.1–0.9 g) of Chromspher UOP RP-18 particles were suspended in 10 ml of buffer consisting of 10 m*M* sodium tetraborate–5 m*M* sodium phosphate–4 m*M* SDS (pH 10). Detection was carried out at 206 nm.

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 and represents the latest method in the field of EKC. In analogy with MEKC, electrokinetic chromatography using suspensions of chromatographic particles as a pseudo-stationary phase will be termed suspension electrokinetic chromatography (SEKC).

The separation of the analytes will be achieved according to the distribution between the buffer and the particle surface. This process leads to a change in the velocity of the separated species. For uncharged species it is necessary to create a relative velocity between the buffer and the particles, otherwise there will be no influence on the analyte velocity and consequently no sepa-

ration. The separation of charged analytes can be influenced either by using charged particles (with their own mobility) or uncharged particles (without their own mobility). In the latter instance the mobility of the charged analyte is decreased by the adsorption process. The greatest influence on the retention time will be found for particles showing an opposite migration direction to the analyte. This effect increases with increasing velocity differences between the analyte and the particles. Charged particles based on classical stationary phases known from pressure-driven chromatography [normal-phase (NP) and reversed-phase (RP) particles are mostly used] with sufficient mobility can be formed according to pH (NP) by the use of surfactants interacting with the polar surface (RP-18), or by synthesizing particles with a certain degree of charged groups.

3.2. Comparison to CZE and related techniques with other pseudo-stationary phases

To achieve a sufficient resolution of the analytes it is necessary to have a large migration-time window. This is defined as the possible range of the migration time of a neutral analyte and is limited between the migration times of the bulk solution (t_0) and that of the pseudo-stationary phase (t_q).

In EKC, the velocities of the analytes and the pseudo-stationary phases such as micelles were described by Hückel [7]. The basis of this application is the fact that the thickness of the diffuse double-layer, δ, (Stern model) is larger than d_p, which is correct for ions and micelles. For the chromatographic particles that we use as the pseudo-stationary phase δ is always smaller than d_p. In this case the particle velocity is described by the equation developed by Smoluchowski [8]:

$$v = \frac{\varepsilon E \zeta}{\eta} \quad (1)$$

where v = velocity of the particle, E = electrical field strength, η = shear viscosity of the buffer, ε = dielectric constant and the zeta potential (ζ) characterizes the electrical potential at the particle surface. As the velocity of the particles increases with increasing zeta potential, it is necessary to chose particles with a high zeta potential. If the particle itself shows only a low potential, it is possible to create a higher one on its surface by coating, e.g. with SDS. The coated particles we use show a zeta potential of 43 mV.

It is unknown whether the influence of a coated particle is caused by the apolar RP phase or by the polar outside. Therefore, only the net effect will be considered. Amphiphilic analytes may be incorporated in the surface layer as described for micelles by Terabe et al. [9]. Usually the concentration of the surfactant used is higher than the critical micellar concentration (cmc), so the effect of micelles, which influence the separation, also has to be considered.

The resolution for uncharged species in EKC is described by the following equation according to Terabe et al. [3]:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)\left[\frac{1 - t_0/t_q}{1 + (t_0/t_q)k_1}\right] \quad (2)$$

where $k_{1,2}$ = capacity factors of analytes 1 and 2, t_0 = retention time of the electroosmotic flow (EOF), t_q = retention time of the pseudo-stationary phase and α = separation factor = k_2/k_1.

The last term on the right-hand side is due to the contribution of the limited migration-time window between the boundaries t_0 and t_q. To obtain an improved resolution it is necessary to decrease the ratio t_0/t_q. Two principal approaches are obvious [9].

From Eq. 2, a relationship among retention times and migration velocities can be developed:

$$v = L/t_r \quad (3)$$

where L = effective length of the capillary, t_r = retention time of the analyte,

$$t_0/t_q = v_q/v_{eof} \quad (4)$$

and v_q = velocity of the pseudo-stationary phase.

It is possible to decrease the electroosmotic flow or to increase the electrophoretic mobility. The electrophoretic mobility may be increased dramatically using particles as the pseudo-stationary phase which show higher velocities

than micelles. As a consequence, the resolution will be improved for a given separation factor. This is obviously an advantage of SEKC over MEKC. The resolution may be optimized by varying either the velocity of the particles or their concentration.

3.3. Peak broadening compared with other electrokinetic techniques

The H/E dependence for MEKC was discussed in detail by Terabe et al. [2] and Sepaniak and Cole [10]. The total band broadening (H_{tot}) is described as the sum of five independent parameters:

$$H_{tot} = H_l + H_m + H_{aq} + H_T + H_{ep(m)} \quad (5)$$

where H_l = longitudinal diffusion, H_m = adsorption/desorption kinetics, H_{aq} = intermicelle mass transfer, H_T = radial temperature gradient and $H_{ep(m)}$ = 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.

The longitudinal diffusion decreases with increasing applied voltage. The influence of H_m and $H_{ep(m)}$ depends on the capacity factor. They both increase with increasing velocity (increase in the applied voltage). For a larger k' and a high v_{eof}, $H_{ep(m)}$ shows the greatest influence, whereas H_m contributes significantly to a medium k' and high v_{eof}. An analogous equation may be derived for SEKC.

For the discontinuous set-up an additional parameter, H_i, has to be considered (see Eq. 6) due to the inhomogeneity of the particles. The parabolic flow profile which results from pumping the particles into the capillary leads to differences in concentrations at the end of the zone. A cross-sectional concentration gradient of the particles is observed. Therefore, the desorption process leads to peak broadening which is serious for large k' and high v_{eof}. $H_{ep(p)}$ is used instead of $H_{ep(m)}$ for the micelles to describe the influence of different particle velocities. For SEKC the total peak broadening is composed of

$$H_{tot} = H_l + H_m + H_T + H_{ep(p)} + H_i \quad (6)$$

where $H_{ep(p)}$ = dispersion due to different particle velocities and H_i = dispersion due to flow profile. For a mixed system (MEKC and SEKC due to the need to suspend the particles), the following equation results:

$$H_{tot} = H_l + H_m + H_{aq} + H_{ep(m)} + H_T + H_{ep(p)} + H_i \quad (7)$$

4. Results and discussion

4.1. Particles suspended in a buffer system containing SDS as surfactant

Particle coating

The main disadvantage of SEKC is the necessity to suspend the particles in the buffer. A more or less stable suspension (according to sedimentation and, therefore, to the size of the particles) may be formed using ultrasonication. As RP particles are not moistened by water, the addition of surfactants such as SDS 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 exterior.

The minimum amount needed to form a suspension was found to be the cmc of the buffer solution. The surface tension of this system was measured using the Wilhelmy plate method. The determination of the cmc is made with a Gibbs diagram (plotting σ versus concentration of SDS), where the cmc is indicated as the minimum of the curve. For the aqueous buffer we used, the cmc was found to be 4 mM for SDS. The resulting suspension remains stable for more than 1 h. The drawback of this method is that the used surfactant acts as an eluent, resulting in low capacity factors.

Assuming about 40 A^2 [11] as the space required for one SDS molecule, only 0.1% of the 4 mM SDS involved is needed to cover all particles in the suspension (0.9 g per 10 ml of

buffer). Therefore, the amount of SDS can be considered to be independent of the particle concentration.

To check this assumption, the amount of SDS left in the buffer was measured. As the electrolyte we used 5 m*M* sodium molybdate. Indirect UV detection was achieved at 211 nm. Fig. 1 shows an electropherogram of 1 m*M* SDS in water. Calibration was carried out with SDS in water in the range 0–2.5 m*M*. The remaining SDS concentration in the particle suspension was measured in the solution after sedimentation of the particles. For the quantification of SDS the solution was diluted (four-fold) to suppress the formation of micelles. The calibration and the amount of SDS found in the solution are given in Fig. 2. This experiment shows that the concentration of SDS in the buffer can be regarded as constant in the presence of the particles.

Particle velocity

The EOF and the mobility of the particles show opposite directions. The velocity of the particles is 17.7 cm/min (at 20 kV) and is larger than the EOF. To avoid problems arising with optical detection (light scattering of the particles), a discontinuous experimental set-up is

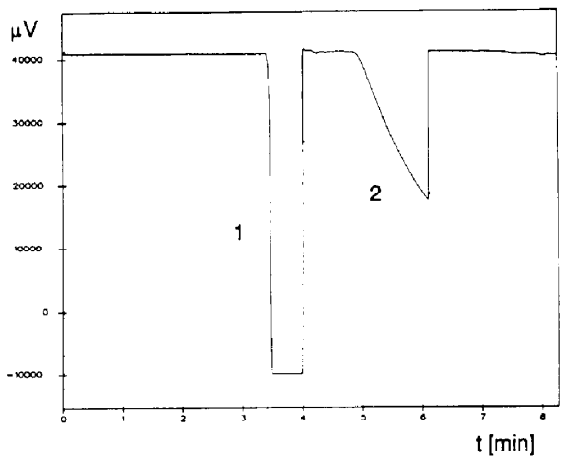

Fig. 1. Electropherogram of SDS. Conditions: electrolyte, 5 m*M* sodium molybdate; capillary, total length 83 cm, effective length 63 cm, 75 μm I.D.; detection, UV at 211 nm, indirect; separation, 30 kV; sample, 1 m*M* SDS; injection, 150 mbar, 0.4 min. Identification: 1 = EOF; 2 = SDS.

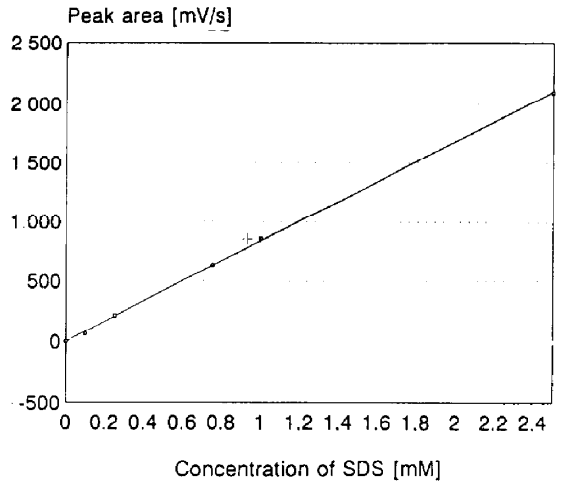

Fig. 2. Concentration of free SDS. □ = Calibration; + = 1.5 μm.

used. The capillary is filled with the suspension up to the detection window. The buffer for electrophoresis consists of the electrolyte and contains the same SDS concentration as the suspension to allow a constant particle velocity. The sample is injected hydrodynamically. The particles which have a higher mobility than the EOF move out of the capillary during the separation while the EOF transports the analytes towards the detection window. The phenol derivatives we used are slower than the EOF in any observed case. Separation is achieved with positive voltage at the injection end. The velocity parameters observed in this SEKC are shown schematically in Fig. 3. This experimental set-up is limited to low capacity factors. Analytes that have a high capacity factor or a high mobility compared with the EOF may be removed from the capillary.

As the degree of coverage of the surface with SDS is high in an aqueous buffer, the influence of the particles on the separation has to be seen as a competition of surfactant, acting as an eluent, and analyte. An additional influence will be found for bifunctional analytes. These analytes may additionally interact with the polar surfactant at the surface or may also be incorporated in the surface layer of SDS comparable to micelles [9].

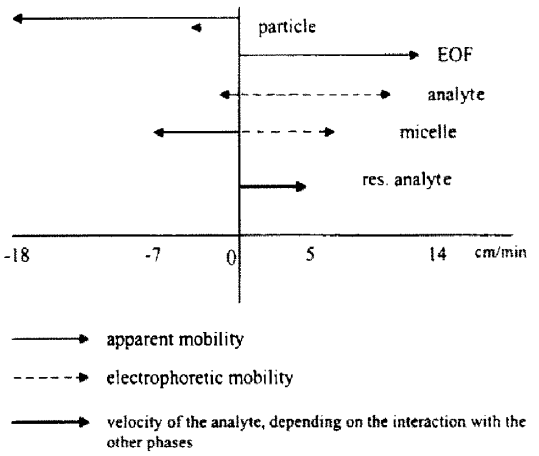

Fig. 3. Velocities of the different phases in SEKC.

Influence on the separation of phenol derivatives

To obtain an impression of the influence of the particles on plate height and to compare the results with those of MEKC and the predictions drawn from theory, we measured the H/E dependence for three phenolic compounds. The resulting data for MEKC compared with SEKC for 4-nitrophenol are given in Fig. 4. A decrease in plate height is found for larger voltages. This may be due to the limited heat dissipation in the particle zone. It is therefore necessary to work at lower voltages compared with MEKC to obtain the best resolution.

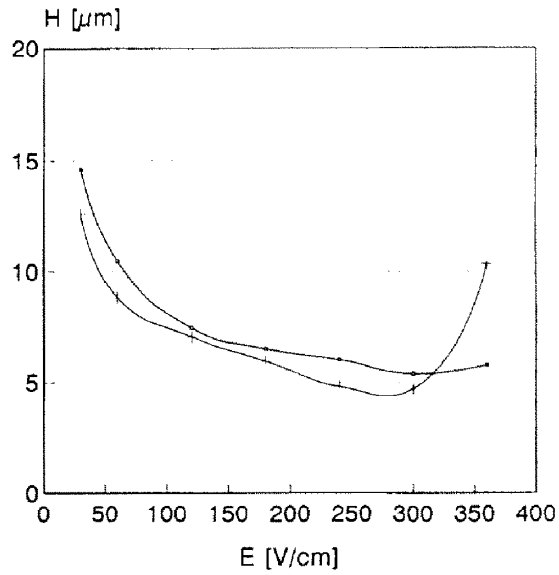

Fig. 4. H versus E curves for 4-nitrophenol. □ = Without particles; + = with particles.

In a further investigation, the influence of different particle concentrations (the buffer contains 4 mM SDS) on the separation of nine phenol derivatives was measured.

Generally, it is possible to separate phenolic compounds according to their own mobility [12–14] or by the use of MEKC [15,16]. We used a buffer system that will not lead to a complete separation. The aim of our work was to show the influence of the particles on a simple separation problem. Phenols were chosen as an example of analytes with low capacity factors. Under the given separation conditions (buffer of pH 9.5), most of the phenols are negatively charged. The pK_a values of the phenols involved are given in Table 1. All the systems investigated consisted of the same buffer and SDS concentration. Fig. 5A shows the separation of nine phenol derivatives in free-flow CZE without SDS. All phenol derivatives are slower than the EOF, so they can be detected in the EOF direction. Some of the phenols are already partly separated.

The influence of SDS needed for the preparation of the suspension was examined. Fig. 5B shows the same separation with an additional content of 4 mM SDS. The resolution of the separation is only slightly affected. As described above, the amount of the micelles is not changed even at increased particle concentrations because

Table 1
pK_a values of the phenols studied

Phenol	pK_a
4-Chloro-3-methylphenol	9.54
2-Chlorophenol	4.50
2,4-Dichlorophenol	7.89
2,4-Dinitrophenol	4.07
2-Methyl-4,6-dinitrophenol	4.70
2-Nitrophenol	7.23
4-Nitrophenol	7.16
Phenol	10.00
2,4,6-Trichlorophenol	6.23

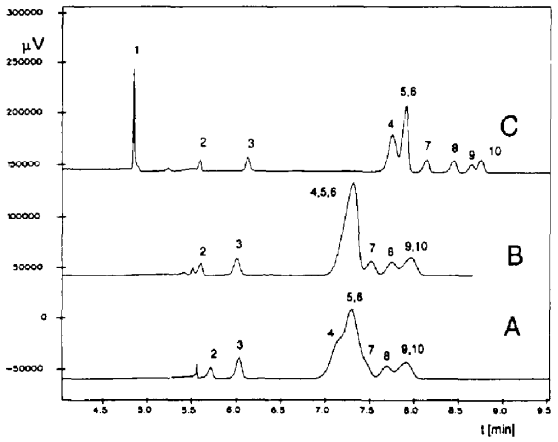

Fig. 5. Comparison of the separation of nine phenols using (A) buffer, (B) SDS and (C) 0.1 g of particles in 10 ml of buffer. Conditions: buffer, 10 m*M* sodium tetraborate–5 m*M* sodium phosphate (pH 9.5); SDS concentration, 4 m*M*; particle amount, 0.1 g of RP-18 (1.5 μm) in 10 ml of buffer with SDS; capillary, 59 cm to detector, 77 cm total length, 75 μm I.D.; injection, hydrodynamic, 50 mbar, 12 s; analyte concentration, 0.9 m*M* of each phenol; separation, 20 kV; detection, UV at 206 nm.

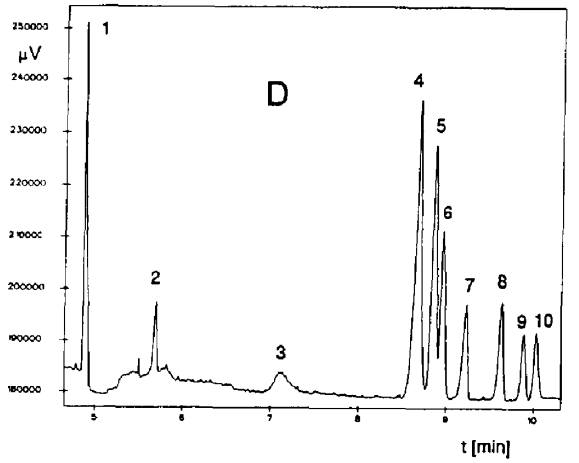

Fig. 6. Identification of the phenol deivatives (separation with 0.9 g of particles in 10 ml of buffer). Peaks: 1 = EOF; 2 = phenol; 3 = 4-chloro-3-methylphenol; 4 = 2,4,6-trichlorophenol; 5 = 2,4-dichlorophenol; 6 = 2-chlorophenol; 7 = 2-methyl-4,6-dinitrophenol; 8 = 2,4-dinitrophenol; 9 = 4-nitrophenol; 10 = 2-nitrophenol.

SDS is used in a large excess. Hence it is possible to compare this electropherogram with the results of the influence of a particle suspension. In Fig. 5C a separation with the aid of a suspension containing 0.1 g of particles in 10 ml of buffer is shown. In Fig. 6 the identification for a particle amount of 0.9 g in 10 ml of buffer for the separation of phenols is given. Analytes adsorbed at the particle surface (independent of the discussed SDS/RP mechanism) show an increased retention time according to the higher mobility of the particles compared with the analytes showing no adsorption. In the case of phenol derivatives an increased resolution is observed. An increase in particle concentration results in longer retention times (Fig. 7).

Calculation of the capacity factor for SEKC

For the calculation of capacity factors for discontinuous SEKC using SDS for particle coating, a simple model was developed. As described above, Eq. 3 gives the relationship between retention time and the velocity of the analyte.

The total velocity of the analyte in MEKC (Eq. 8) and continuous SEKC (Eq. 9) is composed of the velocity present on or in the different phases (micelles, buffer or particle) weighted with the molar fraction ($X_i = n_i/n_{tot}$ where n_i and n_{tot} are the analyte amount in

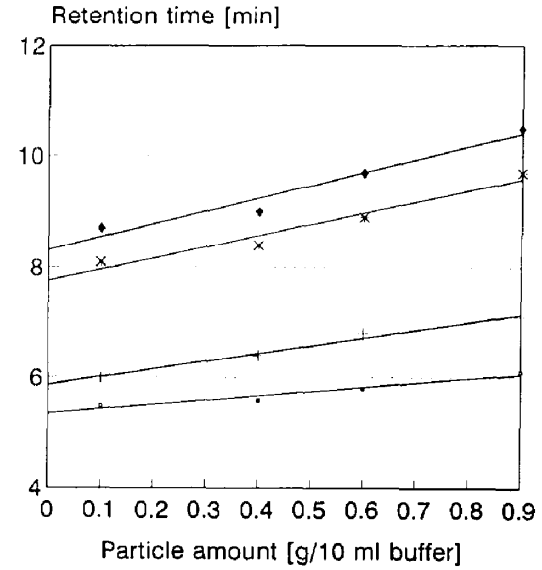

Fig. 7. Influence of the particle concentration on retention time. □ = Phenol; + = 4-chloro-3-methylphenol; * = 2-methyl-4,6-dinitrophenol; ◆ = 2-nitrophenol.

phase i and the total amount, respectively) plus the velocity of the EOF.

$$v_{tot1} = X_1 v_m + X_2 v_a + v_{EOF} \quad (8)$$

where v_{tot1} = velocity of the analyte in MEKC and v_a = velocity of the analyte.

$$v_{tot2} = X_3 v_m + X_4 v_a + X_5 v_p + v_{EOF} \quad (9)$$

where v_{tot2} = velocity of the analyte in continuous SEKC and v_p = velocity of the particles.

The velocity measured for discontinuous SEKC consists of two parts, first the behaviour of the analyte in the particle zone and second of the analyte in the zone without particles (Eq. 10), both weighted with the time fraction they spend in each zone (Eq. 11).

$$v_{tot3} = (v_{tot1})Z_1 + (v_{tot2})Z_2 \quad (10)$$

$$Z_i = \frac{t_i}{t_{tot}} \quad (11)$$

where v_{tot3} = velocity of the analyte in discontinuous SEKC, Z_i = time ratio ($i = 1$, without particles; $i = 2$, particle zone), t_i = time spent in zone i and t_{tot} = retention time of the analyte.

Additionally, two more equations for the mass balance of each system are available for MEKC (Eq. 12) and SEKC (Eq. 13).

$$X_1 + X_2 = 1 \quad (12)$$

$$X_3 + X_4 + X_5 = 1 \quad (13)$$

Further, the balance for the time fractions is

$$Z_1 + Z_2 = 1 \quad (14)$$

As the micelle concentration is not changed by the addition of the particles, which could be proved by the calculation given earlier, the capacity factor of the micelle is constant, leading to

$$k_m = \frac{n_a}{n_m} = \frac{X_1}{X_2} = \frac{X_3}{X_4} \quad (15)$$

where n_a = moles of the analyte in the buffer, n_m = moles of the analyte in the micelles and k_m = capacity factor of the micelles.

The time spent in each zone (Eq. 16) is a consequence of the relative velocity among the analyte and the particles (Eq. 17).

$$t_2 = \frac{v_{rel}}{L_p} \quad (16)$$

$$v_{rel} = v_{tot2} - v_P \quad (17)$$

where v_{rel} = relative velocity and L_p = length of the particle zone. This leads to

$$Z_2 = \frac{L_P}{t_{tot}(v_{tot2} - v_P)} \quad (18)$$

If all values for the different velocity parameters are known, at least seven equations are necessary to obtain the solution due to the seven unknown molar and time fractions.

The velocity of the particles, the micelles, the EOF and the velocity of the analyte are known from direct measurements. According to Terabe et al. [3], Sudan III is used as a tracer for the micelle velocity (for the electrolyte we use a velocity of the micelles of 6.5 cm/min). Hence the calculation of all molar fractions is possible. As the capacity factor k_p is the ratio of the total moles of analyte on the particle surface to those in the surrounding buffer, it is derived as the ratio of the mole fractions of the particle and of the buffer:

$$\frac{X_5}{X_4} = k_p \quad (19)$$

where k_p = capacity factor of the particles. Therefore, it is necessary to calculate both molar fractions. X_1 and X_2 may be calculated first. Using Eqs. 8 and 12, it is possible to obtain

$$X_2 = \frac{v_{tot1} - v_m - v_{EOF}}{v_a - v_m} \quad (20)$$

X_1 is derived from Eq. 12. Combining Eqs. 10 and 18 leads to

$$V_{tot2} = \frac{t_{tot} v_p (v_{tot3} - v_{tot1}) - L v_{tot1}}{t_{tot}(v_{tot3} - v_{tot1}) - L} \quad (21)$$

The combination of Eqs. 10, 13 and 15 leads to

$$X_5 = \frac{(v_a - v_m) - (k_m + 1)(v_{tot2} - v_{EOF} - v_m)}{(v_a - v_m) - (k_m + 1)(v_p - v_m)} \quad (22)$$

Table 2
Capacity factors of the phenols studied at different particle concentrations

Phenol	Capacity factor	
	0.1 g per 10 ml of buffer	0.9 g per 10 ml of buffer
4-Chloro-3-methylphenol	0.03	0.62
2-Chlorophenol	0.17	0.84
2,4-Dichlorophenol	0.17	0.79
2,4-Dinitrophenol	0.21	0.98
2-Methyl-4,6-dinitrophenol	0.19	0.89
2-Nitrophenol	0.25	1.01
4-Nitrophenol	0.20	0.99
Phenol	<0.01	0.18
2,4,6-Trichlorophenol	0.13	0.75

Table 2 summarizes the capacity factors derived from Eq. 19 for the phenol derivatives at given particle amounts of 0.1 and 0.9 g in 10 ml of buffer. As predicted, the capacity factors increase with increasing particle concentration. For the phenols involved they are low. This is caused mainly by two effects: first, SDS acts as a strong eluent, and second, the charged phenol derivatives show little tendency to adsorb on the particles.

For a different system where SDS is not necessary to form a suspension, we expect higher capacity factors and therefore a larger influence of the particles on retention time.

4.2. Reproducibility

The reproducibility for SEKC is found to be comparable to or slightly lower than that for MEKC or CZE. For the retention time and peak area reproducibilities of 1–5% are found.

5. Conclusions

This is the first stage of an investigation concerning the use of SEKC. The aim was to demonstrate some of the possibilities and difficulties resulting from this experimental set-up. It was shown that using SEKC it is possible to influence a separation based on CZE and MEKC. The capacity of the system is controlled by the particle concentration. Therefore, we are able to vary this parameter without affecting others. As the capacity factors are small, it will be a major task to increase the capacity factors in order to obtain increased effects on resolution. An increased effect on the resolution will be found for particles having an opposite migration direction compared with the analytes. This can perhaps be achieved in the case of anionic species such as phenolates by the use of, e.g. tetradecyltrimethylammonium bromide instead of SDS as a surfactant. There is a strong need for the development of particles optimized for SEKC. These particles have to have a partly charged surface and functional groups to interact with the analyte.

The use of other detection methods such as amperometric or fluorescence detection, which are not affected by particles, will help to realize continuous SEKC in the future. This will lead to increased effects on retention and resolution.

Acknowledgement

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