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Review

Application of particles as pseudo-stationary phases in electrokinetic chromatography

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Abstract

Since the introduction of micelles by Terabe et al. (1984), many different species have been employed as carriers for electrokinetic chromatography. As it is not possible to separate uncharged solutes or ions with equal mobility in capillary electrophoresis, these phases are added to introduce or improve selectivity.

This review surveys the application of particles as pseudo-stationary phases. Up to now only very few applications exist based either on polymer particles for the separation of catechols and primary amines or silica-based material. With these particles separations are shown for phenols, polycyclic aromatic hydrocarbons and naphthalene derivatives. The characteristic properties of particles as pseudo-stationary phases and their benefits as well as their limitations are discussed. © 1997 Elsevier Science B.V.

Keywords: Reviews; Pseudostationary phases; Electrokinetic chromatography; Phenols; Polynuclear aromatic hydrocarbons; Naphthalenes

Contents

1. Introduction

During the last two decades, capillary electro-

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phoresis (CE) has expanded into all fields of analytical research. The most promising characteristics are high separation efficiency which is due to the flat flow profile induced by the electric field and short analysis time required for a separation. In CE, separation is achieved due to different electrophoretic mobilities of the analytes in the electric field. Therefore, the separation of uncharged solutes and ions with equal mobilities is not possible. This lack of selectivity has been overcome by the addition of so-called pseudo-stationary phases. These methods represent the connection between electrophoresis and chromatography and are summarized as the field of electrokinetic chromatography (EKC). They all profit from the high efficient separation in short analysis time and high selectivity known from chromatography. Separation in these systems is achieved according to the different distribution coefficients of the solutes between the pseudo-stationary phase and the buffer.

The first electrokinetic method, MEKC, was introduced by Terabe et al. [1]. Micelles of sodium dodecyl sulfate (SDS) were used for the separation of phenols. Since this time, many separations were presented with this system [2]. One problem with the use of micelles is the separation of highly hydrophobic compounds as they are included totally in the micelles and coelute with the migration time of the pseudo-stationary phase. An increase in the affinity of these compounds for the buffer by the addition of organic modifier is rather limited due to the instability of the micelles in hydroorganic phases. This disadvantage also holds for the microemulsions (MEEKC) which consist of oil, water, a surfactant and a co-surfactant and were proposed as pseudo-stationary phase by Watarai [3]. It is obvious that there is a strong need for the development of new phases that are specifically suited for the analysis of very hydrophobic neutral analytes.

Up to now different phases which are stable and soluble in hydroorganic media are known. Palmer et al. [4] employed oligomerized sodium-10-undecylenate (SUA) for the separation of phthalates, PAHs and alkylbenzenes. SUA has a similar structure to micelles but is stable due to the covalent structure. The core is more polar than that of micelles, therefore different selectivity is achieved.

In addition to these micelle-like structures, mac-rocyclic and macromolecular phases have also been applied. Bächmann et al. [5] used native β-cyclodextrin (β-CD) for the separation of polycyclic aromatic hydrocarbons (PAHs). In this case mobility of the carrier is achieved due to deprotonation of secondary hydroxy groups at high pH. Alternatively the charge can be introduced in CDs by derivatization of the hydroxy groups which was shown for the separation of neutral compounds [6]. Separation is achieved according to diffent stabilities of the inclusion complexes of the analytes and the CDs. With these chiral selectors even the separation of neutral enantiomeric compounds was possible (CD–EKC) as demonstrated by Schutzner and Fanali [7].

A promising pseudo-stationary phase are the resorcarenes. These macromolecules are characterized by high stability in hydro-organic buffer systems and high charge and therefore high mobility in the electric field. They have been successfully applied for the separation of PAHs [8]. High efficiencies were achieved due to the lack of microheterogeneity of these macromolecules. As selectivity is introduced via the functional groups during synthesis a broad range of selectivity can be created. Limitations for the application of these macromolecules are given by the high UV absorptivity due to the resorcinol units.

Other kinds of carriers which are stable in the full range of water–organic solvent mixtures are the starburst dendrimers introduced by Tanaka et al. [9]. These monospheric species are more hydrophobic than the micelles. Due to the easy derivatization, they are useful for investigating structure-dependent effects of pseudo-stationary phases [10].

Each of the presented pseudo-stationary phases has extended the applicability of CE to the analysis of various types of compounds. Since most of these phases have not been used in chromatographic systems, it is not possible to predict and adjust the selectivity to a given separation problem which would be preferable in order to solve separation problems easier and quicker.

A very promising approach in this direction is the use of chromatographic particles as pseudo-stationary phases because knowledge about selectivity drawn from chromatography can be adapted to electrophoresis. The applicability of EKC should be increased due to the broad selectivity spectrum of these phases. Any selectivity can be adjusted using

mixtures of different particles. New selectivities can be introduced in EKC compared to micelles and the other phases.

With respect to HPLC and capillary electrochromatography (CEC), this application implies a kind of chromatography based on a one-run column. Therefore problems concerning reconditioning and ageing of the columns are avoided. In this field even the use of particles in the nanometer size is possible which gives high capacity due to the large surface area. In analogy to MEKC, the use of suspensions of particles is called SEKC.

Up to now the field of particle application is very small, only three different types of particles have been employed. The applications with these particles and the basic investigations made with these systems are presented in this review.

2. Fundamentals

2.1. Requirements for particles

The characteristic features of particles which are suitable as pseudo-stationary phases in electrokinetic chromatography are basically the same as for the other phases described above.

They should:
- form homogeneous dispersions in any buffer system (this also includes highly organic media)
- provide selectivity in the interaction with the solutes
- have a charge to provide electrophoretic mobility
- show equal mobility to prevent peak broadening
- have a small mass transfer resistance for the analytes to prevent peak broadening
- not disturb the detection
- be small to provide a high surface for the interaction and to avoid light scattering.

The particle core can either be of polymer or inorganic material with a diameter smaller than 1 μm. The interaction with the analytes can either take place with the core or a group which is bonded to it. This group may, for example, be a long alkyl chain for hydrophobic interaction. For the mobility of the particles, the surface must provide charges or chargeable groups like sulfuric acids or carboxylic acids or their ester compounds. Generally the introduction of

this charges can be achieved by dynamic coating or chemical bonding. To avoid high detector noise the particle should not consist of UV active groups.

2.2. Calculations

In CE and EKC, the velocities of the analytes and the pseudo-stationary phases like micelles are calculated as described by Hückel [11]. This calculation is based on the fact that the thickness of the diffuse double-layer δ, (Stern-model) is larger than the particle diameter d_p, which is suitable for ions and small molecular aggregates like micelles. For the chromatographic particles in the nanometer range, δ is always smaller than d_p. In this case the particle velocity is described by Eq. (1) developed by von Smoluchowski [12].

$$v = \frac{\varepsilon \cdot E \cdot \zeta}{\eta} \tag{1}$$

In this equation v is the velocity of the particle, ε is the dielectric constant, E is electric field strength, and η represents shear viscosity of the buffer.

The zeta-potential (ζ) characterizes the electrical potential at the particle surface. As the velocity of the particles increases with increasing ζ-potential, it is necessary to choose particles with a high ζ-potential. If the particle itself shows only a low potential, it is possible to create a higher potential on its surface and therefore a higher mobility by coating, for example, with sodium dodecyl sulfate.

2.3. Peak broadening in particle systems

In EKC the optimum separation efficiency is determined by the so-called H/E plot where the plate height (H) is correlated to the applied electric field strength (E). For MEKC this dependence is discussed in detail by Terabe et al. [13] and Sepaniak and Cole [14]. Total band broadening (H_{tot}) is described as the sum of five independent parameters, Eq. (2).

$$H_{tot} = H_l + H_m + H_{aq(m)} + H_T + H_{ep(m)} \tag{2}$$

The meaning of the individual magnitudes run as follows. H_l-longitudinal diffusion, H_m-adsorption/desorption kinetics, $H_{aq(m)}$-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 be applied to SEKC. For pure SEKC, the total band broadening is composed of

$$H_{tot} = H_l + H_m + H_{aq(p)} + H_T + H_{ep(p)} \qquad (3)$$

where $H_{aq(p)}$ is the interparticle mass transfer and $H_{ep(p)}$ represents dispersion due to different particle velocities.

3. Applications of particles

3.1. Polymer particles

Particles were introduced as pseudo-stationary phases in electrophoresis by Wallingford and Ewing [15]. They employed a monodisperse sulfonated polymer (Eastman AQ 55S) with a diameter of about 20 nm. The particles consist of a hydrophobic core and a charged outer sphere of sulfonated groups. Therefore they are negatively charged at the chosen pH of 7.08. In comparison to micelles these particles are stable in hydroorganic buffer systems. The separation of five catechols was achieved as shown in Fig. 1. Efficiency decreased with increasing retention time. Broader bands were found in comparison to MEKC. Nonetheless, Wallingford and Ewing [15] predict unique properties of monodispersed polymers that may open a new area of research in the field of EKC.

The second application for these polymer particles and the investigation of their electrophoretic properties have been made by Palmer et al. [16]. Since the particles are prepared from isophthalic acid, isophthalic acid-5-sulfonate (sodium salt), diethylene glycol and 1,4-cyclohexanedimethanol UV detection of the solutes is not possible below 350 nm due to the high background. For the investigation of the particle velocity UV detection was carried out at 254 nm. Acetone was used as EOF marker. The migration time window was found to increase with increasing amount of methanol from 4.1 min (100% water) to 14 min (60% water/40% methanol). In
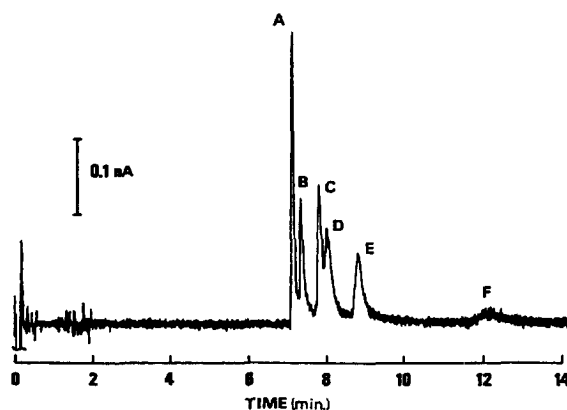

Fig. 1. Separation of catechols using monodispersed polymer beads. Conditions: Electrolyte: 5 mM sodium hydrogen phosphate, 5 mM sodium bihydrogen phosphate, pH = 7.08; Suspension: 1% (w/v) Eastman AQ 555 polymer; Separation: 20 kV; Capillary: 0.65-m effective length, I.D. 26 μm; Identification: A = hydroquinone, B = catechol, C = 4-methylcatechol, D = 3-methylcatechol, E = 4-ethylcatechol, F = 4-tert.-butylcatechol (80 μM each). (Taken from Ref. [15] with permission).

every case the particle signal was broad implying that the polymer dispersion consisted of particles with different electrophoretic mobilities. Buffer with 40% acetonitrile yielded discouraging results.

The use of polymer particles as pseudo-stationary phase for electrokinetic chromatography was investigated for the separation of some derivatized alkyl amines and polycyclic aromatic hydrocarbons (PAHs). The running buffer consisted of 60% buffer and 40% methanol. Detection was achieved at 350 nm. Under these conditions no selectivity was observed for the employed PAHs benzo[ghi]perylene, fluoranthene and benz[e]acephenanthrene. All three compounds eluted at the same time which was found to be the migration time of the particles.

The separation of the nitrobenzofurazan (NBD) derivatives of propyl-, pentyl-, and heptylamine as well as aminofluorene was successfully done with a Beckman PACE 2100. Detection was achieved using laser-induced fluorescence with an argon ion laser. Excitation was made at 488 nm and emission at 520 nm. The polymer dispersion contained a concentration of 0.1% (w/v) of the particles. The retention order was found to be NBD-pentylamine, NBD-heptylamine, NBD-aminofluorene and NBD-propylamine with curiously the least hydrophobic solute

eluting last. The efficiencies varied widely for the analytes. With increasing hydrophobicity and increasing retention time a decrease in efficiency was found from 130 000 down to 4500 plates. For NBD-propylamine, an increase in efficiency was found to 90 000 plates. Palmer et al. concluded that the interaction mechanism with this polymer is analyte specific therefore it was difficult to predict and interpret the results.

3.2. Silica based particles

3.2.1. Reversed-phase material

The aim of the application of dynamically coated particles was to demonstrate that reversed-phase material can be used as pseudo-stationary phase in EKC. Therefore any selectivity known from chromatography should be available for electrokinetic separations. Since these particles do not possess any charge, the application in SEKC is only possible after dynamic coating of the surface. This is done with surfactants in concentration at or higher than the critical micellar concentration (CMC). Investigations with reversed-phase particles were made by Bächmann et al. [17] using RP-18 material. As demonstrated in Fig. 2, the surfactant interacts with the hydrophobic chains of the reversed-phase particle, creating a charged outer sphere. This surface modification is well known from micellar liquid chromatography (MLC) [18] where surfactants are used to prevent high consumption of toxic organic eluents. Fig. 2 also describes the different equilibria between the analyte (phenol) and the buffer, the micelles and the particles, respectively.

Due to the coating, the particles are moistened in aqueous buffers and show very high electrophoretic mobility. In an aqueous buffer system, a ζ-potential of around 43 mV was found for these particles which means that particle mobility is higher than the EOF mobility.

The first application with these particles was done for the separation of phenol derivatives. Due to the mobility of the phenols, some of the analytes were already separated in the aqueous buffer as demonstrated in Fig. 3A. The influence of the micelles, which are present in this buffer system due to the dynamic coating, is given in Fig. 3B. Resolution was only slightly affected in comparison to the pure
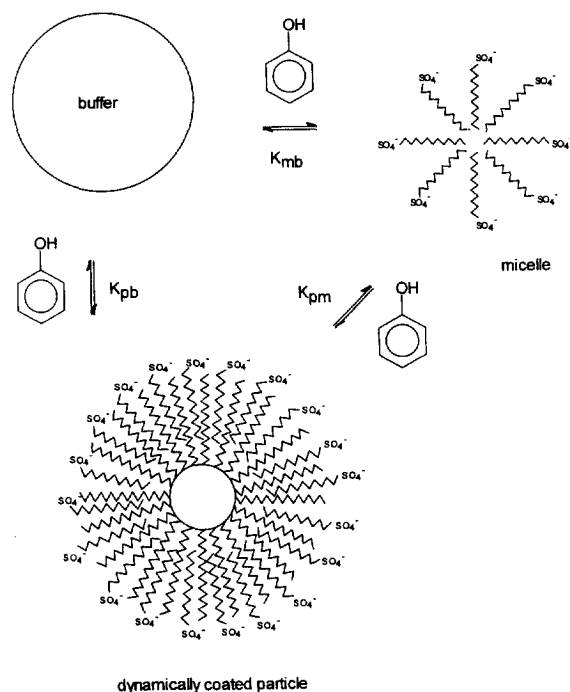

Fig. 2. Interaction mechanisms in SEKC with dynamically coated particles.

buffer. Increased resolution was found for the application of the suspension as shown in Fig. 3C. Complete separation was achieved due to the employment of 5% (w/v) particles as given in Fig. 4.

Another example for the application of reversed-phase material was given for the separation of PAHs [19]. These solutes were chosen due to their very hydrophobic character. Normally, solubility of highly hydrophobic compounds is enhanced by the addition of high amounts of organic modifiers. In case of dynamically coated particles, this is not possible due to the instability of the coating since the organic molecules replace the surfactant molecules [18] creating an apolar surface which leads to agglomeration of the particles and therefore to instability of the suspension. This fact is obviously a disadvantage of this system. Nevertheless is was possible to increase solubility of the PAHs with the addition of urea and β-CD. The influence of the micelles can be neglected due to the small amount of SDS used in this electrolyte. Therefore, no separation was found with the buffer system as demonstrated in Fig. 5. The
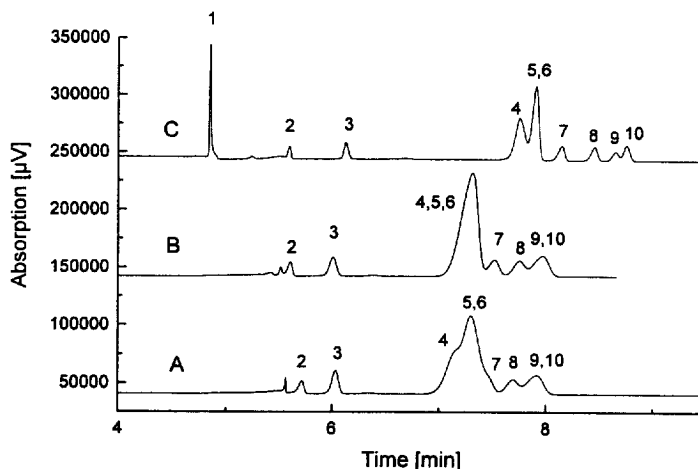
Fig. 3. Comparison of the separation of nine phenols using buffer (A), SDS (B) and suspension (C). Conditions: Electrolyte: 10 mM sodium tetraborate, 5 mM sodium phosphate, pH = 9.5; B: electrolyte A with 4 mM SDS; C: electrolyte B with 0.5% (w/v) reversed-phase particles, d_p = 1.5 μm; Injection: hydrodynamically 50 mbar, 12 s, separation: 20 kV; Capillary: 0.77-m total length, 0.59-m effective length, I.D. 75 μm; Detection: UV at 206 nm; Identification: 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. (Taken from Ref. [17] with permission).

successful separation of four PAHs due to the application of reversed-phase particles is also given in Fig. 5.

3.2.2. Chemically modified silica

In order to overcome the problem of dynamic coating and to broaden the applicability of SEKC, new particles were created which are stable in

hydroorganic media [20]. In the first step the silicasurface was silylated with 10-(carbomethoxy)-decyldimethylchlorosilane. The charge is created in the second step, when ester hydrolysis takes place. The procedure for the production of the particles is schematically shown in Fig. 6. Due to the chemical bonding of the charged group these particles are stable in buffers containing high amounts of organic
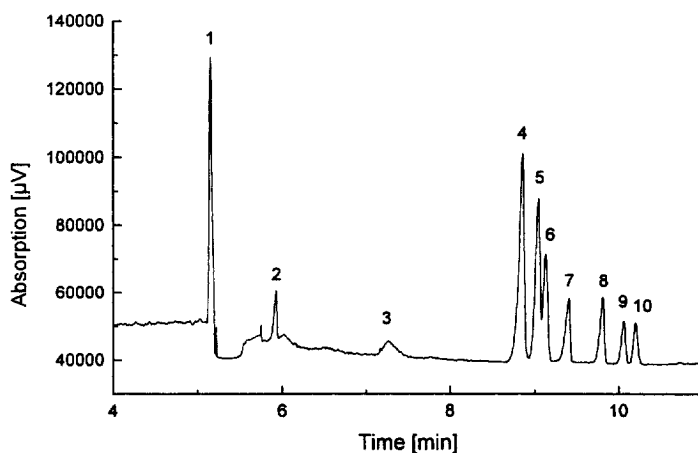

Fig. 4. Separation of nine phenol derivatives with 5% (w/v) particles. Other conditions as described in Fig. 3. (Taken from Ref. [17] with permission).
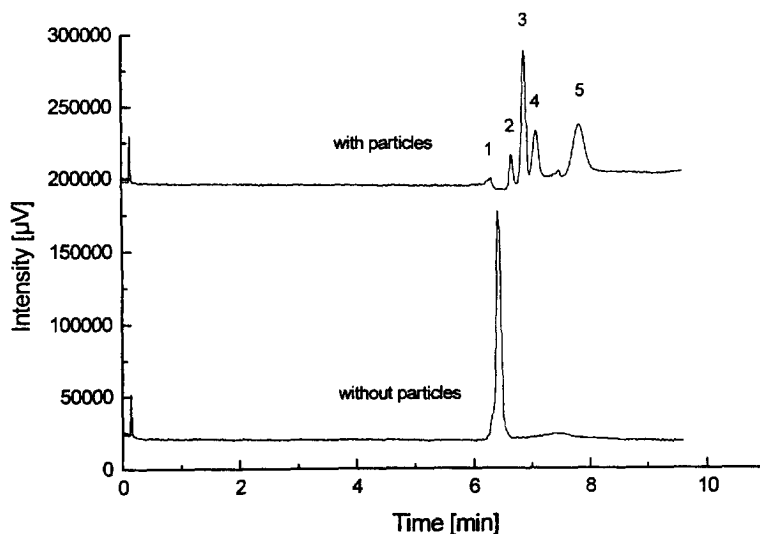
Fig. 5. Separation of PAHs with reversed-phase particles (dynamic coating); Conditions: Electrolyte: 10 mM sodium tetraborate, 5 mM sodium phosphate, 4 mM sodium dodecyl sulfate, 4 M urea, 40 mM β-CD, pH=9.5; Suspension: 3% (w/v), reversed-phase, d_p =500 nm; Injection: electrokinetic 10 s, 10 kV; Separation: 30 kV; Capillary: 1-m total length, 0.7-m effective length, I.D. 75 μm; Detection: fluorescence, ex. 245 nm, em. 280 nm, cut-off; Identification: 1 =EOF, 2 =naphthalene (12.5 ppm), 3 =anthracene, 4 =phenanthrene, 5 =2,3-benzofluorene (5 ppm each). (Taken from Ref. [19] with permission).
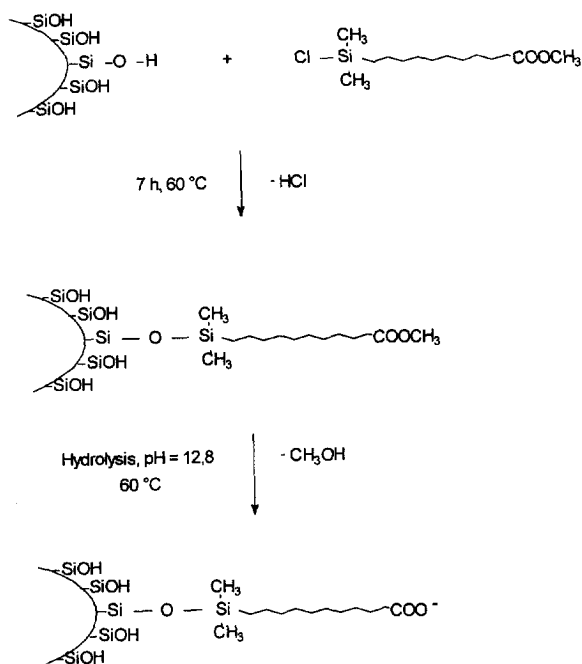

Fig. 6. Silylation of the silica particles. (Taken from Ref. [20] with permission).

solvents. The successful separation of some PAHs is shown in Fig. 7.

In HPLC, selectivity has been thoroughly investigated. It is concluded that molecular size, shape and hydrophobicity are the dominant factors controlling retention in the reversed-phase mode. For PAHs, the interesting properties are the aromatic system (correlation factor, F) [21], the size (molecular connectivity index, c, and the van der Waals volume, W_v) [22,23] and the hydrophobic character which is given by the distribution coefficient in a mixture of 1:1 octanol:water (P) [21]. In case of a reversed-phase-like retention mechanism the capacity factors of the analytes show linear dependencies with the parameters described above.

For the separation of PAHs with these new designed particles, selectivity was found to be comparable to HPLC. This was also proved by the plot of log k' versus log P as given in Fig. 8 which shows linear dependence (correlation factor 0,993). Separation efficiency was found to decrease with increasing retention. This was already recognized with the other particle systems [15].

The observed band broadening in SEKC was investigated for the separation of naphthalene deriva-
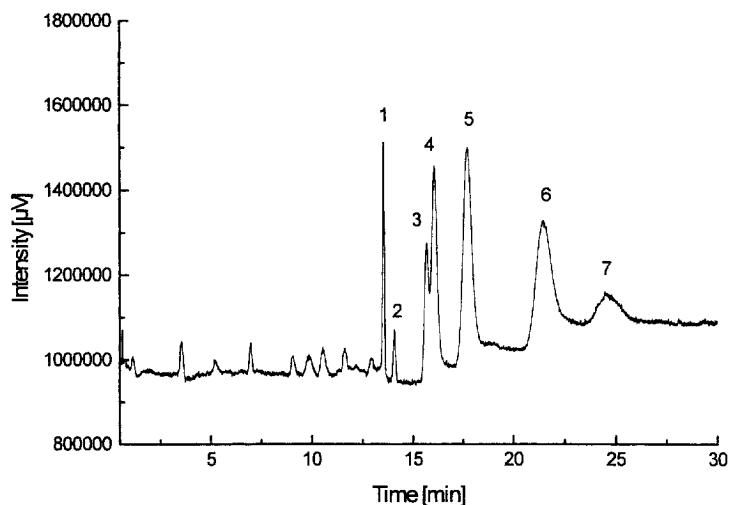
Fig. 7. Separation of PAHs with silylated particles. Conditions: Electrolyte: 5 mM sodium tetraborate, 2.5 mM sodium phosphate, 8 M urea, 50% methanol, pH=7.0; Suspension: 5% (w/v) carboxydecyl-derivatized particles, d_p=500 nm; Injection: hydrostatically 30 s, 10 cm; Separation: 30 kV; Capillary: 1-m total length, 0.65-m effective length, I.D. 75 μm; Detection: fluorescence, ex. 245 nm, em. 280 nm, cut-off; Identification: 1=7-Amino-4-methylcumarin (2.5 ppm), 2=naphthalene (10 ppm), 3=phenanthrene (2.5 ppm), 4=anthracene (2.5 ppm), 5=pyrene (2.5 ppm), 6=2,3-benzofluorene (2.5 ppm), 7=benzo[a]pyrene (2.5 ppm). (Taken from Ref. [20] with permission).

tives (Fig. 9). To examine the factors that determine efficiency in SEKC the plate heights of the different compounds were investigated for different applied voltages. The H/E plot is shown in Fig. 10. Different curve shapes were found for the marker compound (DMSO, investigated parallel in UV-detection) and the analytes.
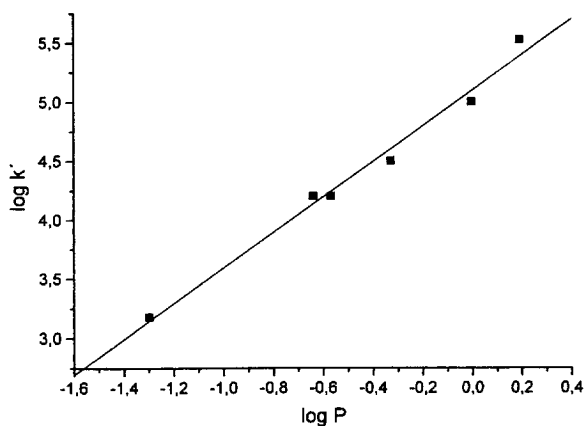

Fig. 8. Plot of log k' versus log P for the separation of polycyclic aromatic hydrocarbons (for actival separation compare Fig. 7). (Taken from Ref. [20] with permission).

DMSO itself showed no interaction with the particles. Concerning band broadening this compound suffered from all parameters except $H_{aq(p)}$ and $H_{ep(p)}$.

The increase of plate height with increasing field strength for DMSO was attributed to insufficient heat dissipation in the capillary. This effect was proved by the Ohm plot where the current is plotted against the applied voltage. For the buffer system used, deviation from linearity was found above 20 kV.

As is obvious from the H/E plot in Fig. 10, inverse curve shapes were found for the other analytes. These shapes were related to the insufficient heat dissipation at higher electric field strengths. In these cases, the higher temperature leads to decreasing mass transfer resistance of the analytes on the particle surface. This kind of mass transfer resistance is well known from micellar liquid chromatography (MLC) [18] and is a result of the charges at the particle surface. The decrease in mass transfer resistance showed the highest effect for compounds with high capacity factors. The capacity factors of the analytes were calculated as described by Terabe et al. [24] and are summarized in Table 1. The migration time of the particles was 48 min.
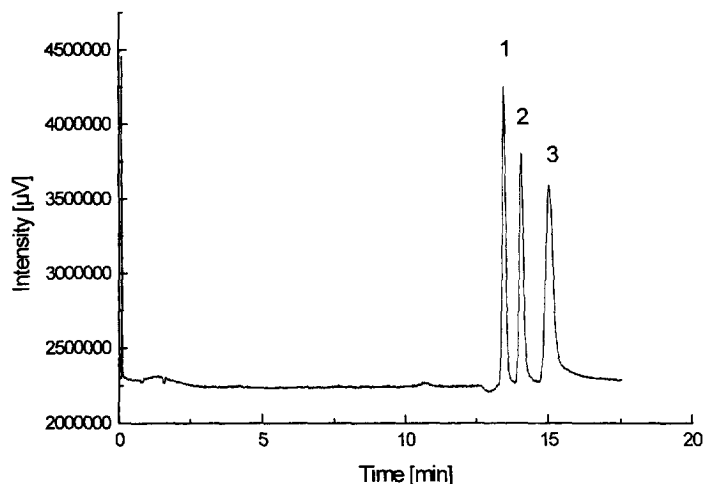
Fig. 9. Separation of naphthalene, 2-methylnaphthalene and 2,6-dimethylnaphthalene. Conditions: Electrolyte: 5 mM sodium tetraborate, 2.5 mM sodium phosphate, 50% methanol, pH = 7.0; Suspension: 5% (w/v) carboxydecyl derivatized particles, d_p = 500 nm; Injection: hydrostatically 30 s, 10 cm; Separation: 30 kV; Capillary: 1-m total length, 0.65-m effective length, I.D. 75 μm; Detection: fluorescence, ex. 219, em. 280 nm, cut-off; Identification: 1 = naphthalene, 2 = 2-methylnaphthalene, 3 = 2,6-dimethylnaphthalene, 20 ppm each. (Taken from Ref. [20] with permission).
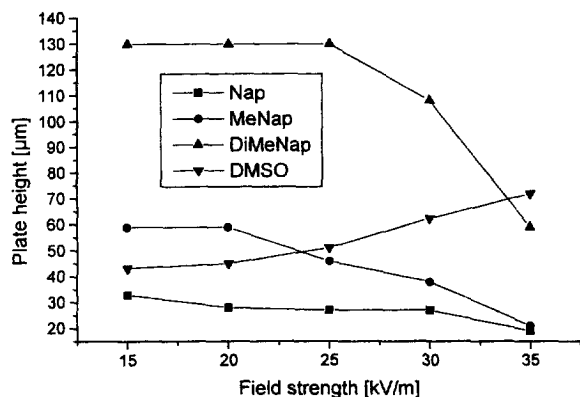

Fig. 10. Dependence of plate height on applied field strength for naphthalene derivatives and DMSO as marker. Nap = naphthalene, MeNap = 2-methylnaphthalene, DiMeNap = 2,6-dimethylnaphthalene. (Taken from Ref. [20] with permission).

Table 1
Capacity factors of the analytes

	k'
DMSO	0
Naphthalene	0.07
2-Methylnaphthalene	0.15
2,6-Dimethylnaphthalene	0.31

2,6-Dimethylnaphthalene which had the highest capacity factor showed the greatest decrease in mass transfer resistance.

Another characteristic for EKC is the increasing peak broadening with increasing capacity factors. This effect had already been observed by Szolar et al. [25] who used a mixture of two uncharged and two charged β-CDs to separate PAHs. Decreasing plate numbers with increasing retention time were attributed to different mobilities of the CDs due to different substitution for the charged CD.

Different velocities may also be found for the particles. Since the particle velocity is not dependent on the particle size, this can only be due to different surface composition, either different coating or different hydrolysis of the ester groups. Fig. 11 shows the comparison of the baseline increase in UV absorption for a zone of particles and the EOF marker. In case of the EOF, a sharp step was observed whereas in the case of the particles, a smooth increase took place which is a consequence of different particle velocities.

For naphthalene and 2-methylnaphthalene, an increase in efficiency is found compared to the marker. This is due to the lack of particles in the sample zone. Therefore the capacity factors of the
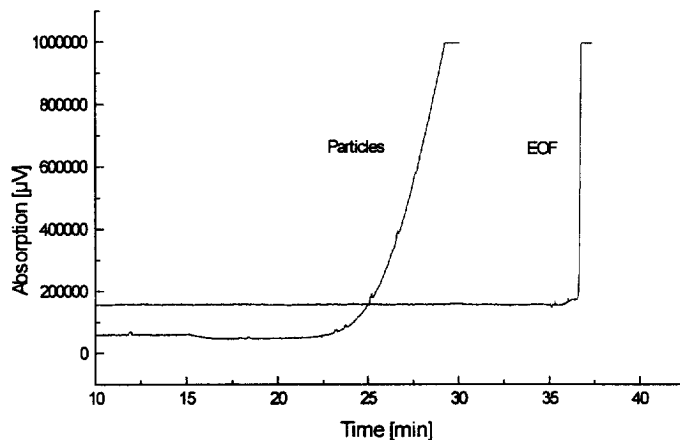
Fig. 11. Increase of UV absorption of particle zone and EOF marker. Conditions: Electrolyte: 5 mM sodium tetraborate, 2.5 mM sodium phosphate, 50% methanol, pH = 7.0; Suspension: 5% (w/v) carboxydecyl-derivatized particles, d_p = 500 nm; Capillary: 0.65-m total length, 0.15-m effective length, I.D. 75 μm; Separation: 4 kV; Injection: as bulk; Detection: UV, 220 nm. (Taken from Ref. [20] with permission).

analytes are zero. Under the applied field the pseudo-stationary phase migrates into the sample zone. Analyte focussing occurs in this zone due to different capacity factors. This means that the zone of the analytes gets sharper. Therefore separation efficiencies of the analytes should be better than for the marker and higher with increasing capacity factors. In the case of the separation of the naphthalene derivatives, the H/E plot demonstrates this effect for naphthalene and for 2-methylnaphthalene at temperatures where mass transfer resistance is reduced. Both compounds show smaller plate heights than DMSO. For the other derivative peak broadening due to mass transfer resistance counteracts the focussing.

4. Conclusion

This review showed different applications of particles as pseudo-stationary phases in electrokinetic chromatography. Successful separations were made for catechols, phenols, primary amines, PAHs and naphthalene derivatives.

Polymer particles have proved to be a suitable pseudo-stationary phase for hydrophilic compounds like catechols and primary amines but no separation was possible for more hydrophobic compounds.

In the case of silica based material, two different approaches were made. One for reversed-phase

particles which have been successfully employed for phenol derivatives and PAHs. Limitations are given for the addition of organic modifiers to these particle systems. Nevertheless these kind of particles are interesting due to the possibility of adjusting selectivity known from chromatography to electrophoresis. The application of any known chromatographic phase is possible using dynamic surface coating. This might for example be useful in the case of chiral analytes.

The second application of silica based material was done with especially modified particles. This led to phases which were stable in any electrolyte system, even with high organic amounts. Successful separations were demonstrated for PAHs and naphthalene derivatives. Selectivity was found to be comparable to HPLC. Up to now efficiencies (normally in the range of 1 million plate numbers/m) with this system were not as high as expected due to the heterogeneity of the particle surface which is due to the production. This process has further to be optimized.

Limitations for all particle separations are given by the heterogeneity of the ζ-potentials which lead to different mobilities and therefore to loss in efficiency. In case of the modified silica material increase in efficiency can be realized by preparation of the suspension. In free flow electrophoresis the suspension can be fractionized due to their mo-

bilities. Due to the lack of UV active groups and decreased light scattering this system is suitable for UV detection. Therefore we think that SEKC, with newly designed particles, is an upcoming method in the field of EKC.

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