ELSEVIER

Journal of Chromatography B, 783 (2003) 113–124

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Microemulsion electrokinetic chromatographic analysis of some polar compounds

Heli Sirén[a,b,*], Anne Karttunen[a]

[a]*Technical Research Centre, VTT Processes, P.O. Box 1602, FIN-02044 VTT Espoo, Finland*
[b]*Laboratory of Analytical Chemistry, P.O. Box 55, University of Helsinki, FIN-00014 Helsinki, Finland*

Received 6 September 2001; received in revised form 19 July 2002; accepted 19 July 2002

Abstract

This study presents the optimization of a microemulsion electrokinetic chromatographic (MEEKC) electrolyte solution by using UV detection and with the method, simultaneous separations of chemically, biochemically and pharmaceutically related anionic and cationic compounds. Representatives of the compound groups were from isoflavonoids, benzodiazepines, metanephrines, diuretics and peptide hormones. The MEEKC separations under basic conditions were first optimized using a two-component isoflavonoid mixture as the sample and an electrolyte containing 10 m*M* tetraborate as the main buffer (pH 9.5). The stable microemulsion phase was adjusted with various amounts of octane, 1-butanol and sodium dodecyl sulfate (SDS). An only acidified electrolyte solution used in the study was made of phosphoric acid (pH 1.8) containing octane, SDS and ethyl acetate. The analyses with isoflavonoids showed that electrophoretic mobilities of the investigated compounds were highly related to the concentrations of SDS and 1-butanol with linear and parabolic correlation, respectively. However, addition of octane gave linear correlation only at low concentrations. In most cases four to six structurally related compounds and even 13 diuretics with various polar properties were separated from each other in basic microemulsion medium. The acidified MEEKC electrolyte gave good resolution for anionic metanephrines.

Keywords: Microemulsion electrokinetic chromatography; Polar compounds

1. Introduction

Microemulsion electrokinetic chromatography (MEEKC) is an electrophoresis technique related to micellar electrokinetic chromatography (MEKC). It has been shown to be extremely powerful especially in the separation of pharmaceuticals [1–3].

Microemulsions are heterogeneous liquids, which are made optically transparent and thermodynamically stable. To keep microemulsions soluble they are made of water, water-soluble organic solvent, charged surfactants and co-solvents. Organic microemulsion droplets are stabilised to the aqueous phase by surfactants and organic water-soluble co-solvents. Usually MEEKC solutions are mixtures of sodium dodecyl sulfate (SDS) to enhance separation between sample compounds, an alkane to form microemulsion droplets in the aqueous solution, some alkyl alcohol to reduce the surface tension

*Corresponding author. Laboratory of Analytical Chemistry, P.O. Box 55, University of Helsinki, FIN-00014 Helsinki, Finland. Tel.: +358-91-915-0264; fax: +358-91-912-0253.
E-mail address: heli.siren@helsinki.fi (H. Sirén).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00519-6

between the alkane and water phases [1–4]. Because the MEEKC electrolytes are made of reagents having low-UV absorptivities at wavelengths below 230 nm, they can be used as media for on-line detection of low-UV-absorbing compounds.

Separation of compounds in MEEKC is based on both compound partitioning between the microemulsion droplets and the water phase, as well as on their electrophoretic mobilities [1,2,4]. Differences in the selectivity of MEEKC electrolytes can be obtained by using different surfactants and by changing their concentrations [5–8]. Manipulation of the microemulsion phase influences the separation of the compounds [1,2,5–9]: polar compounds favour remaining in the aqueous electrolyte solution rather than partition into the microemulsion droplets. As for neutral compounds, they move with both the electroosmotic flow (EOF) and the microemulsion droplets in water and organic phases, respectively. Cationic analytes with pK_a values higher than the pH of the microemulsion solvent can form ion-pairs (IPs) with the anionic SDS surfactant layer on the emulsion droplet. Probably, they may even totally adsorb into the microemulsion droplets. If ion-pair formation is to be excluded, cationic sample compounds in the presence of anionic surfactants are better separated in uncharged or co-charged microemulsion phases to exclude the IP effect. Ionized acidic drugs and macromolecules have been shown to have very low affinity towards the anionic surface molecules, but as far as we know the role of nonionic MEEKC has not been thoroughly studied. When the pH of the MEEKC electrolyte solution remains below 5, silanol groups of the capillary surface are partly protonated and the EOF is lower than the electrophoretic mobility of the microemulsion droplets. That is the case in low-pH MEEKC solutions, where reverse polarities are used to fasten the analyses [8].

The common trend in, e.g., clinical and doping laboratories is to analyze as many structurally related compounds as possible in one run. In our study mixtures of isoflavonoids, benzodiazepines, catecholamines, diuretics, peptide hormones and proteins were used as the samples. Isoflavonoids, which have a structure of C_6–C_3–C_6 flavone, have an oxygen and three carbons to form a heterogenous ring in the molecule. Flavonoids and their metabolites are found in plants as *O*-glycosides and in human body fluids as *O*-glycosides, *O*-ramnosides and sulfates. The most popular technique to analyse flavonoids has been high-performance liquid chromatography (HPLC) with electrochemical detection (ED) [10].

Benzodiazepines are aromatic 1,4-benzodiazepine adducts, of which ca. 20 are in pharmaceutical use. They have an anxiolytic effect on central nerve systems and are abused by drug addicts. Their phase I and II metabolites [11] are most commonly screened from blood and urine samples by enzyme-multiplied immunoassay, with HPLC or after derivatization with gas chromatography (GC) [12].

Catecholamines have a 1,2-hydroxybenzene structure and one or more amine and/or carboxylic acid functions. Some of them are formed in the sympathetic nervous system and cortex of humans. Catecholamines and their phase I and II metabolites, so-called metanephrines, are interesting, since they diagnose pheochromocytoma and neuroplastoma, Parkinson's disease and stress diseases and can be found in body fluids. Due to the low concentrations and various metabolites they are mainly analysed from body fluids with HPLC using ED. Recently, capillary zone electrophoresis (CZE) has also shown potential in the quantification of cationic catecholamines [11,13].

Diuretics are used in medication to promote excretion of body fluids and salts. In addition, they are used for lowering blood pressure [14–16]. Diuretics vary in mechanisms of operation and duration of their efficacy, and hence have clinical use. In a chemical respect, they are a challenging group of compounds in MEEKC studies, since diuretics are acids, bases and neutral compounds [16,17]. A group of diuretics have earlier been successfully analysed with CZE and MEKC [17–19], but not with MEEKC.

Proteins, peptides and amino acids are essential components of all living organisms. Due to the cationic and anionic functional groups in the analytes and, e.g., low-UV absorption, their analysis without derivatization is a difficult task. They can be screened by HPLC, capillary electrophoresis (CE) or high-resolution (HR) GC using various kinds of detectors [20–24]. A microemulsion medium can be used for the separation of macromolecules like proteins. Compounds having both acid and base properties were chosen for this study to find out

whether the MEEKC method is capable of their separation. However in this case, the large molecules are not able to partition in the core phase of the microemulsion droplet, but only on its surface. In spite of that, the analysis is more possible than in MEKC medium, where the large macromolecules cannot penetrate the micelles.

Our aim in the present study was to optimise an MEEKC method for screening of some polar anionic and cationic drug compounds, which are used in medical treatment of patients (benzodiazepines) or are monitored in doping control (diuretics). The reasons for choosing the compound mixtures were: (1) to find a specific MEEKC system and (2) to study the performance of the MEEKC technique in drug screening. In addition, as far as we know, our chosen compounds have not been previously researched with MEEKC.

2. Experimental

2.1. Materials

All the following chemicals were obtained from Sigma (St. Louis, MO, USA): metanephrines: 4-hydroxy-3-methoxymandelic acid (VMA, M_r 198.18 g/mol, pK_a values 16.9, 2.3 and 9.9), 3-hydroxy-4-mandelic acid (i-VMA, M_r 198.18 g/mol, pK_a values 16.9, 2.3 and 9.9), 3,4-dehydroxyphenylacetic acid (DOPAC, M_r 168.1 g/mol, pK_a values 4.4, 9.7 and 10.3) and 4-hydroxy-3-methoxyacetic acid (HVA, M_r 182.17 g/mol, pK_a values 4.4 and 7.9), catecholamines: 3,4-dihydroxyphenylalanine (DOPA, M_r 153.14 g/mol, pK_a values 9.6, 2.3, 9.6 and 12.5) and 5-hydroxyindoleacetic acid (5-HIAA, M_r 191.2 g/mol, pK_a values 15.6, 9.9 and 4.5), benzodiazepines: nitrazepam (M_r 281.26 g/mol, pK_a values 2.88 and 10.88), lorazepam (M_r 321.16 g/mol, pK_a values 1.3 and 11.5), diazepam (M_r 284.76 g/mol, pK_a 3.3), and flurazepam (M_r 387.89 g/mol, pK_a 1.4), isoflavonoids: 7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (daizein, M_r 254.25 g/mol) and 5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (genistein, M_r 270.24 g/mol), diuretics [14–16]: metyrapone (M_r 226.3 g/mol), caffeine (M_r 194.19 g/mol, pK_a~14), triamterene (M_r 253.3 g/mol, pK_a 6.2), clopamide (M_r 345.9 g/mol, pK_a 9.1), chlorthalidone (M_r 338.8 g/mol, pK_a 9.3), etacrynic acid (M_r 303.2 g/mol, pK_a 3.5), probenecid (M_r 285.4 g/mol, pK_a 3.4), metyrapone (M_r 226.3 g/mol), bumetanide (M_r 364.4 g/mol, pK_a 5.2), bendroflumethiazide (M_r 421.4 g/mol, pK_a 8.5), trichlormethiazide (M_r 380.7 g/mol, pK_a 8.6), benzthiazide (M_r 432.0 g/mol), hydrochlorothiazide (M_r 297.7 g/mol, pK_a values 7.9 and 9.2), dichlorphenamide (M_r 305.2 g/mol, pK_a 8.2), chlorthiazide (M_r 295.7 g/mol, pK_a 6.7; 9.5) and acetazolamide (M_r 222.3 g/mol, pK_a values 7.2 and 9.5). In addition to those, SDS (purity 99%) and cytochrome *c* (purity 99%) were from Sigma. Protein Test Mix (histamine, lysozyme, cytochrome *c* and ribonuclease A) and Small Molecule Test Mix (EOF marker, nicotine, *N*-acetylprocainamide and procainamide) were obtained from Beckman (Fullerton, CA, USA). Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$), orthophosphoric acid (purity 85%) and thiourea were obtained from Merck (Darmstadt, Germany). Octane (purity 99.5%) was from Fluka (Buchs, Switzerland). Butanol was from Riedel-de Haen (France). Methanol was from Mallinckrodt (Nanograde; Paris, KY, USA). All reagents were of analytical grade.

2.2. Instrumentation

A Beckman-Coulter MDQ (Beckman Instruments, Fullerton, CA, USA) with a UV detection system set at 200 nm was used. The silica capillaries were 60.2 cm (50 cm to the detector)×50 μm I.D.×365 μm O.D. and were obtained from Composite Metal Services (The Chase, UK). The applied voltages were either +20 or +18 kV, unless stated otherwise. The separation temperature was always +40 °C. The samples were injected by a pressure of 0.5 p.s.i. (1 p.s.i.=6894.76 Pa) for 5 to 20 s. The sample tray temperature was maintained at +25 °C. The buffer reservoirs were at ambient temperature. Experiments in tetraborate solutions were carried out in the cationic mode (cathode at the outlet) and those made in the phosphate solution in the reverse mode.

A Denver pH meter Model 20 (Denver Instruments, Denver, CO, USA) was used for pH measurements. The pH combination electrode was calibrated using commercial standards with pH values of 4.00,

7.00 and 10.00 (Radiometer, Copenhagen, Denmark). Pure water was made daily with a Milli-Q water purifying system (Millipore, Avondale, PA, USA).

The ionization of the different functional groups of benzodiazepines and some of the diuretics was estimated with the aid of the pK_a values. The pK_a values of the functionalities were based on predictions of the Pallas 1.2 program (CompuDrug Chemistry, Budapest, Hungary).

2.3. Conditioning of the capillary

New capillaries were washed for 8 min with aqueous 0.1 *M* NaOH solution and for 5 min with Milli-Q water. Before each run they were rinsed with the electrolyte solution for 7 min.

2.4. Preparation of microemulsion solutions

The basic microemulsion solutions ($n=35$) were prepared by weighing the reagents into volumetric flasks. The concentrations of SDS, 1-butanol and octane were 3–5.3%, 6–8.6% and 0.2–1%, respectively. The 10 m*M* sodium tetraborate solution (pH 9.5) was added into the weighed amounts of mixtures containing appropriate amounts of SDS, 1-butanol and octane. The purified water was boiled for 20 min and cooled to +25 °C before use in the preparation of the 10 m*M* tetraborate solution [25].

Only one acidified microemulsion solution was used. It was prepared from 1 ml of orthophosphoric acid in 296.63 ml of Milli-Q water, which was boiled as described above. The pH of the solution was 1.8. The solution was ultrasonicated for 15 min and temperated to +25 °C before use. In acid MEEKC separation ethyl acetate was used instead of 1-butanol. The electrolyte solution was prepared as follows: 3.3% SDS, 6.6% ethyl acetate, 0.8% octane and 89.3% phosphoric acid solution.

All microemulsion solutions were sonicated for 15 min before use. Before sonication they were filtered through 0.45-μm PTFE membranes (Millipore, Molsheim, France). After the MEEKC electrolytes were mixed, they were ultrasonicated for 30 min and cooled to +25 °C before use.

2.5. Electrolyte solutions in basic MEEKC separations

The solution combinations in optimizing the separation contained 10 m*M* tetraborate solution, SDS, 1-butanol and octane at 86.43–89.73% (v/v), 3.05–6.26% (w/v), 6.07–8.49% (v/v) and 0.22–1.01% (v/v), respectively. The final MEEKC solution was evaluated by analysing the drugs with 89.2% (v/v) 10 m*M* tetraborate solution, 3.05, 4.15, 5.22 and 6.26% (w/v) SDS, 6.07, 6.61, 7.58 and 8.49% (w/v) 1-butanol and 0.22, 0.46, 0.61, 0.81 and 1.01% (w/v) octane at pH 9.5.

2.6. Preparation of samples

Stock solutions of the standard compounds were made to concentrations of 20–1000 mg/l. The final sample mixtures for MEEKC studies were prepared from these stock solutions to the concentrations needed and diluted with the microemulsion solvent used in the analyses. When necessary, samples were also filtered through 0.45-μm membranes (Millipore).

Catecholamines and metanephrines were made from 1000 mg/l stock solutions and diluted to 7.5–25 mg/l mixtures for analysis. Benzodiazepine standards were prepared from 90.9 mg/l solutions to 15–30 mg/l mixtures with MEEKC solvent dilution. Isoflavonoids were diluted from 1000 mg/l stock solutions with the appropriate MEEKC electrolyte solution to a 12.5 mg/l mixture and derivatives of acetic acid to a 25 mg/l mixture. The working standard mixtures of diuretics were prepared by mixing 50 μl of the intermediate standard mixture (100 mg/l, mixed by diluting the individual 1000 mg/l stock solutions), 30 μl of 0.1 *M* methanol and 180 μl of the MEEKC solution. Thiourea was used at a concentration of 10 mg/l to mark the EOF of basic solutions in the electropherograms, except in the separation of diuretics, where methanol was used as the marker.

3. Results and discussion

The optimisation of the basic MEEKC electrolyte solution was done by adjusting the concentrations of

SDS, 1-butanol and octane in the tetraborate (98%, v/v). The final chemical composition of the electrolyte was chosen from 35 components of MEEKC solutions (pH 9.5 in all). One acidified microemulsion solution was used: instead of tetraborate it contained phosphoric acid (pH 1.8). The electrophoretic separation of daizein and genistein in different microemulsion media were used to optimize the suitable basic MEEKC solution (Fig. 1, Table 1). Optimisation was directed by the insolubility of octane in the aqueous micellar solvents and also by both the critical micelle concentration (CMC) of SDS (8.1 m*M* in pure water, 25 °C) [18]. The only MEEKC electrolyte with low pH was used in the studies of the catecholamine and metanephrine mixture (Fig. 2) and that of the histidine, lysozyme, cytochrome *c* and ribonuclease A mixture (Fig. 3, Table 2).

3.1. Effect of SDS

In the MEEKC solutions the CMC of the SDS surfactant was not exceeded and therefore the hydrophobic interactions between the analytes and the emulsion surface were weak [11]. The studies showed that anionic SDS had a great effect on partitioning of isoflavonoids (Fig. 1) between the microemulsion and the water phases, although anionic compounds had a low partition into the microemulsion droplets due to their low affinity. The increase of SDS concentration decreased the electrophoretic mobilities of the test compounds: a linear correlation was obtained between 1.2 and 6.5% of SDS in the electrolyte (daidzein: $y=-3.7362x+10.938$, $R^2=0.9891$, Table 1). The electrolyte solution was shown to have a lesser influence on selectivity than on resolution of the catecholamines and metanephrines (Fig. 4) [26]. However, the molecular geometry of the compounds might play a significant role, when the pK_a values of analytes with similar dissociation are of equal magnitude.

Peak broadening in the electropherograms was not observed when the SDS concentration increased by 3–6.3% (w/v) in the MEEKC electrolyte. The studies showed that the change of SDS concentration between the electrolytes was so minor, that the EOF was the same in every test as expected, when the ionic strength is equal between the solutions. However, the higher the SDS concentration in the microemulsion solution, the higher the plate numbers obtained (daizein 87 000/m and genistein 21 000/m). A minor disadvantage of a high SDS concentration was that it gave rise to duplicate peaks in the electropherograms with pressure injections of 5–20 s.

Microemulsions were stable for months, when their SDS concentrations were between 1 and 3.5%. However, they were opal at concentrations below 1% of SDS. The maximum SDS concentration useful in our studies was 2.5% (w/v). On the contrary, higher SDS concentrations would be favourable due to high *N* values of the analytes (Table 1).

3.2. Effect of 1-butanol

In MEKC organic modifiers are added to the buffers to alter migration and to improve resolution. Also, in MEEKC the role of organic modifiers is to make the solution more efficient for separation. When long-chain alcohols are used they affect the polarity, viscosity and density of the electrolyte, increasing affinity of the analytes between the SDS monomers. In this MEEKC study the increase of 1-butanol concentration in the basic microemulsion solutions increased the migration of the test compounds (Fig. 1, Table 1) and improved the analyte zone resolution. Its role here was to improve the stability of microemulsion decreasing the surface tension between the phases. The microemulsion was stable, when 1-butanol concentration was between 6.0 and 8.6% (w/v). The results showed that the relation between the electrophoretic mobilities of isoflavonoids and the concentration of 1-butanol was parabolic (daidzein: $y=10.52x^2-33.329x+32.303$, $R^2=0.9952$).

3.3. Effect of octane

The addition of octane to the electrolyte did not affect the migration of the analytes as noticed elsewhere [27]. It was chosen for the organic modifier, since the carbon skeleton of it and 1-butanol gives the sum of the length of SDS [28]. Increase of octane slightly decreased the electrophoretic mobilities of the isoflavonoids (Fig. 1, Table 1) when

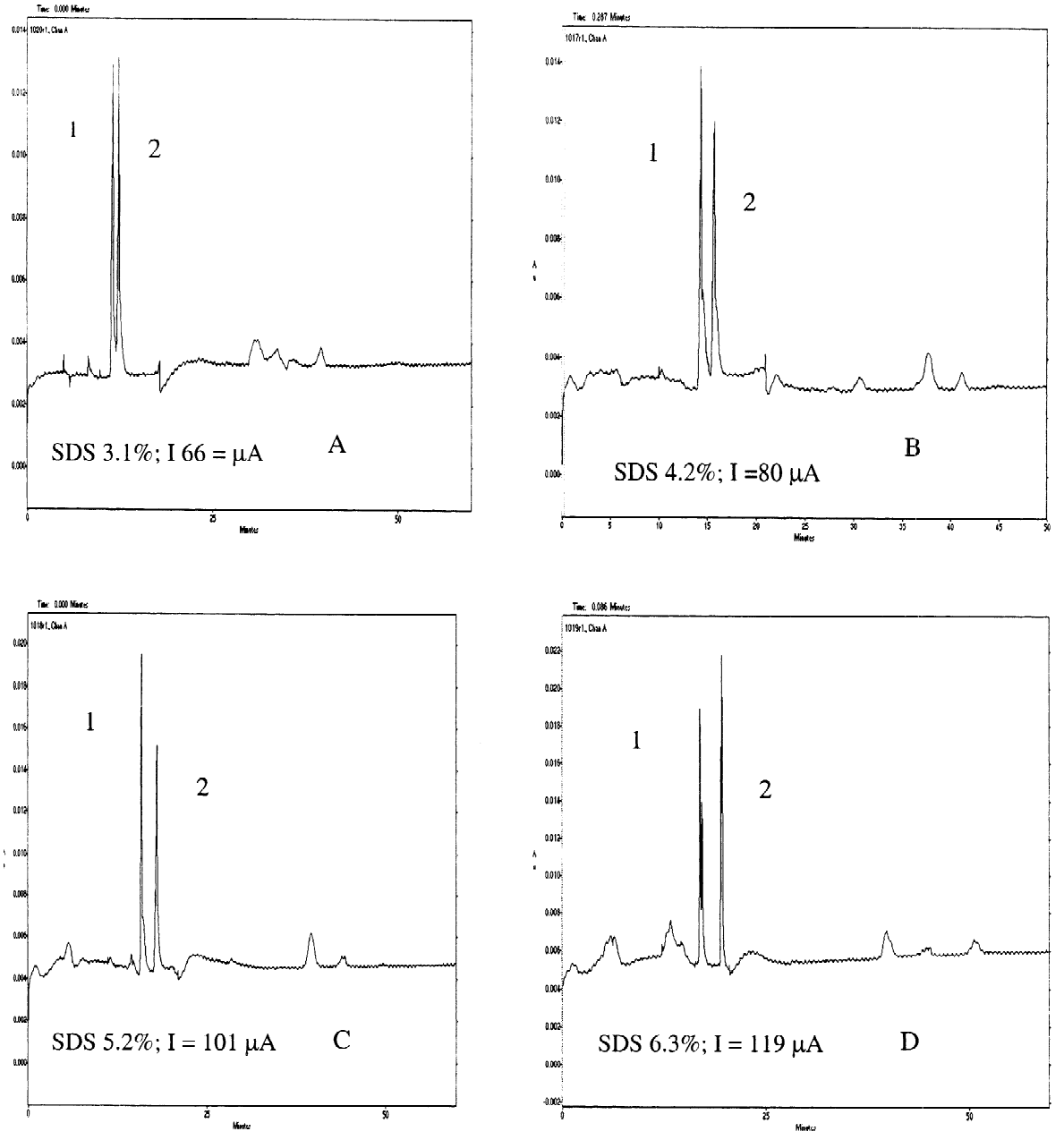

Fig. 1. Separation of isoflavonoids in basic (pH 9.5) microemulsion solution. (A) SDS 0.3069 g, 1-butanol 0.6712 g and octane 0.0893 g; (B) SDS 0.4200 g, 1-butanol 0.6673 g and octane 0.0895 g; (C) SDS 0.5317 g, butanol 0.6599 g and octane 0.0883 g; (D) SDS 0.6446 g, 1-butanol 0.6650 g and octane 0.0880 g. Peaks (1) daizein (M_r 254.24 g/mol) and (2) genistein (270.24 g/mol). Concentration of the analytes 10 mg/l. $L_{det/tot}$=50/60.3 cm; voltage, 20 kV; wavelength, 200 nm; injection with 0.5 p.s.i. for 5 s.

compared to those values obtained in the SDS optimisation at 0.89% octane.

However, addition of octane gave a linear correlation only at low concentrations. Between 0.22 and 0.61% of octane the linear correlation between electrophoretic mobility and the concentration of

Table 1
Electrophoretic mobilities (μ_e) and plate numbers (N) of daizein in the solutions

Electrophoretic mobility ($\cdot 10^{-8}$ m^2 s^{-1} V^{-1})	Plate number, N (1/m)	Effect of SDS (%) (1-butanol 6.6%, octane 0.89%, tetraborate 89.4%)
2.14	9420	3.05
1.76	11 990	4.15
1.54	10 740	5.22
1.27	87 000	6.26
		Effect of 1-butanol (SDS 3.3%, octane 0.87%, tetraborate 89.7%)
1.53	18 400	6.07
1.82	22 500	6.61
1.18	16 500	7.58
1.10	27 400	8.49
		Effect of octane (SDS 3.3%,butanol 6.6% and tetraborate 89.4%)
1.98	34 500	0.22
1.93	20 800	0.46
1.89	24 400	0.61
2.12	24 600	0.81
1.95	22 900	1.01

The most stabile combination for the MEEKC solution was 3.3% SDS, 6.6% 1-butanol, 0.9% octane.

octane were noticed. With larger amounts the addition of octane showed no regular effect on migration of polar compounds. Microemulsions were stable, when the octane concentrations were between 0.2 and 2.95% (w/v). In MEEKC solution, over-concentration of one or more of the reagents showed immediately as an opal solvent.

3.4. Separation of analytes

The anionic compounds migrated according to their increasing molecular mass and to their hydrophobicity. Acids are negatively charged and generally well solubilised in the high pH of the microemulsion buffer. The final basic MEEKC electrolyte for the analyses of isoflavonoids (Fig. 1), benzodiazepine (Fig. 2) and proteins (Fig. 3) contained 3.3% SDS, 6.6% 1-butanol, 0.9% octane and 89% of 10 m*M* sodium tetraborate (pH 9.5). The isoflavonoids migrated according to the increase of molar masses. The structure of genistein having three OH groups instead of two had more affinity to the microemulsion droplet than daizein.

Benzodiazepines (Fig. 2) did not migrate according to their increasing molar masses [29]. Neither had any correlation between their dissociation (pK_a values) and migration velocities. But, it was noticed that those compounds having a secondary amine group in the structure migrated first, followed by those benzodiazepines having a tertiary amine substituted with alkane groups. However, the migration order was the same in the acid MEEKC electrolyte as in the 10 m*M* ammonium acetate (pH 4.0)–acetonitrile solution (90:10, v/v) [28]. However, the difference between the results was the migration window, which was 9 min (migration between 3 and 12 min in the 50.2 cm capillary) and 10 min (Fig. 4, migration between 23 and 33 min in the 60.3 cm capillary) in CZE using a 50% MAPT-coated capillary [30] and MEEKC in a fused-silica capillary, respectively. As already noted before, the peak shapes were essentially better in MEEKC, although the migration times were long and the difference between the currents was 10-fold, 6 and 62 μA in CZE and MEEKC, respectively.

Proteins, being cations in acidic solutions, could be separated with the acid MEEKC electrolyte. The reason that they did not form ion-pairs with the

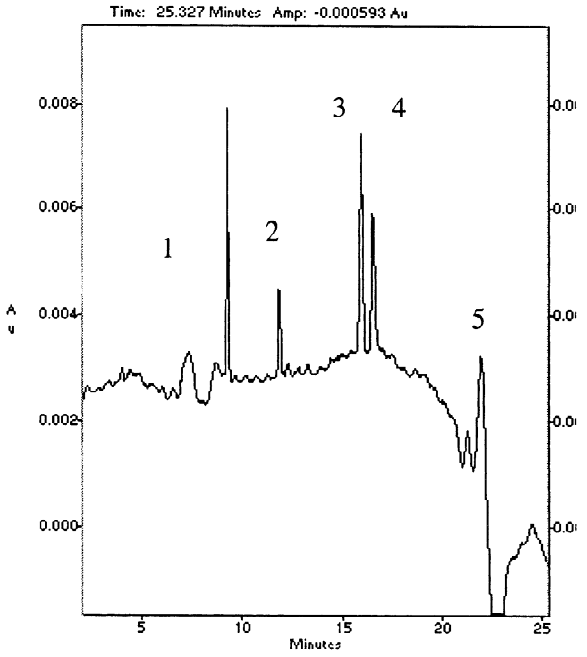

Fig. 2. Separation of benzodiazepines with basic MEEKC solution. Compounds (1) nitrazepam, (2) lorazepam, (3) diazepam and (4) flurazepam. Analyte concentrations are 50 mg/l. Experimental conditions: $L_{det/tot}$=50/60.3 cm; voltage, 20 kV; current, 62 μA; wavelength, 200 nm; injection with 0.5 p.s.i. for 5 s.

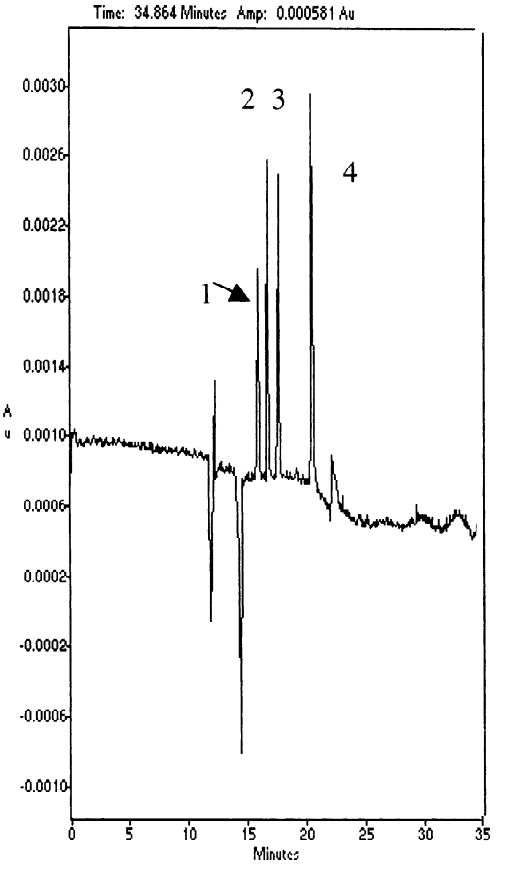

Fig. 3. Separation of protein test mix in acidic MEEKC solution (current, −28 μA). Compounds: (1) histamine, (2) lysozyme, (3) cytochrome *c* and (4) ribonuclease A. injection with 0.5 p.s.i. for 5 s; voltage, −10 kV.

surfactant, was the nonionic surface of the microemulsion phase. Good separation was performed between 15 and 21 min without peak tailing. Detection limits for the proteins were 1–5 mg/l (0.5 p.s.i., 5 s injection). However, in the presence of SDS, cationic catecholamines as well as some proteins probably formed ion-pairs with the anionic SDS molecules on the microemulsion droplets and retain in the electrolyte system. Detection limits of the sample compounds were below the mg/l level for most of the compounds.

Metabolites of catecholamines (Fig. 4), i.e., anionic VMA, HVA and 5-HIAA could only be separated from each other in the acid microemulsion solution. Their migration order was based on their functionality: compounds having an OH group in the molecule migrated before those substituted with a methoxy group. In addition, the molar masses seemed to have some effect on the migration order: compounds with low masses migrated first. However, in the presence of SDS, cationic catecholamines as well as some proteins probably formed ion-pairs with the anionic SDS molecules on the microemulsion droplets and were retained in the electrolyte system.

The most suitable separation electrolyte for diuretics (Fig. 5) contained 3.3% SDS, 7.5% 1-butanol and 1.0% octane in 10 m*M* tetraborate solution (pH 9.5). Our studies showed that the tested MEEKC solvents are more universal for the separation of different kinds of analyte groups than the MEKC solutions used in our earlier studies [16,19]. The migration widow in 50 m*M* glycine–42 m*M* SDS solution (pH 10.5) in MEKC with a 67 cm fused-silica capillary was 13 min (from 7 to 21 min), and for the MEEKC electrolyte (pH 9.5) with a 60.3 cm

Table 2
Electrophoretic mobilities of some compounds studied

Compound	Electrophoretic mobility ($\cdot 10^{-8}$ m^2 s^{-1} V^{-1})	Plate number, N (1/m)
i-VMA[a]	6.90	119 400
DOPA[a]	2.81	68 600
VMA[a]	2.36	177 700
HVA[a]	1.76	52 400
5-HIAA[a]	1.70	62 200
DOPAC[a]	1.26	36 000
Furosemide[b]	−3.19	40 200
Daizein[b]	−3.34	13 200
1,2-Diphenylacetic acid[b]	−3.44	18 400
Genistein[b]	−3.47	15 300
Nitrazepam[b]	−3.86	59 400
Triphenylacetic acid[b]	−3.93	24 400
Lorazepam[b]	−4.11	43 400
Diazepam[b]	−4.15	34 500
Flurazepam[b]	−4.18	71 300

[a] SDS–ethyl acetate–octane–phosphoric acid (10 m*M*, pH 1.8) (3.3:6.6:0.8:89.3).
[b] SDS–1-butanol–octane–sodium tetraborate (10 m*M*, pH 9.5) (3.3:6.6:0.9:89).

capillary it was 24 min (from 12 to 36 min). Partition to the micelles was supposed to be a more molecule-specific effect than partition on the oil droplet surface, since the separation efficiency was better in MEKC separation.

Resolution between the compounds increases, when their migration window widens in MEEKC, which is shown with the amine compounds. Fig. 6 shows the separation of nicotine, *N*-acetyl-procainamide and procainamide. Although their mobility is slow the peaks in the electropherogram are quite narrow (*N* 90 100/m, 24 000/m and 78 000/m in

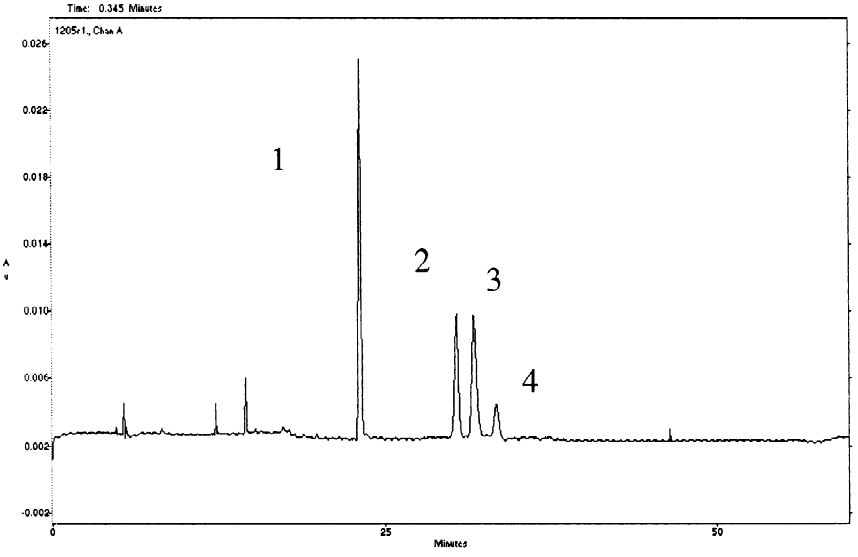

Fig. 4. Separation of anionic catecholamines and metanephrines in acid (pH 1.8) microemulsion solution. Peaks: (1) DOPA, (2) VMA, (3) HVA, (4) 5-HIAA and (5) DOPAC. Analyte concentrations are 5 m*M*. Experimental conditions: L_{det}/l_{tot}=50/60.2 cm; wavelength, 200 nm; voltage, −18 kV; current, −83 μA; injection with 0.5 p.s.i. for 10 s.

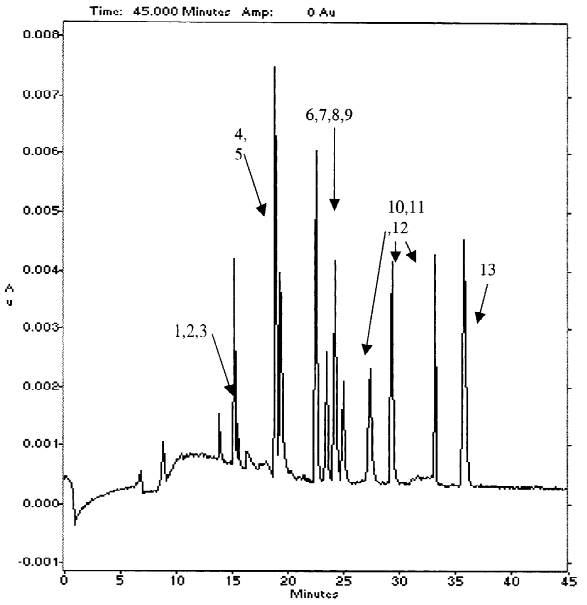

Fig. 5. Separation of diuretics with basic MEEKC electrolyte. Compounds in migration order: 1=caffeine, 2=clopamide, 3=probenecid, 4=bumetanide, 5=bendroflumethiazide, 6=etachrynic acid, 7=trichloromethiazide, 8=hydrochlorothiazide, 9=benzthiazide, 10=metyrapone, 11=triamterene, 12=chlorothalidone, 13=acetazolamide. Current, 35 μA; voltage, +10 kV; detection at 200 nm; $L_{det/tot}$=50/60.3 cm; temperature, +40 °C. Analyte concentration: 10 mg/l. Injection with 0.5 p.s.i. for 5 s.

their migration order, respectively). In MEKC separation usually the peaks are very wide and the mobilities of the compounds are correlated: the slower is the mobility, the broader the peaks are and the lower plate numbers can be calculated. In MEEKC the effect of tetraborate concentration on the separation was not studied, although it was known that the capacity factors would increase at low borate concentrations and stabilise at high concentration [31]. However, increase of the ionic strength should be advantageous, since it decreases the repulsion of borate and surfactant complexes.

Because dissociation and hydrophobicity influence the mobilities of ionic compounds, they also affect their migration behaviour. Furthermore, the overall hydrophilicity/hydrophobicity will affect migration, especially when the interactions with micelles or MEEKC droplets are only hydrophobic: cytochrome *c* migrated in basic MEEKC solution as a nice peak (Fig. 7). Although in MEEKC the microemulsion droplets do not contain the buffering reagent, like in MEKC, but organic solvent, they have due to their large size and different chemistry, more dissolving ability than micelles in MEKC. Our studies showed that diuretics migrate in microemulsion technique more slowly than in MEKC, but their partitioning into the surfactant appears to be equal to that in

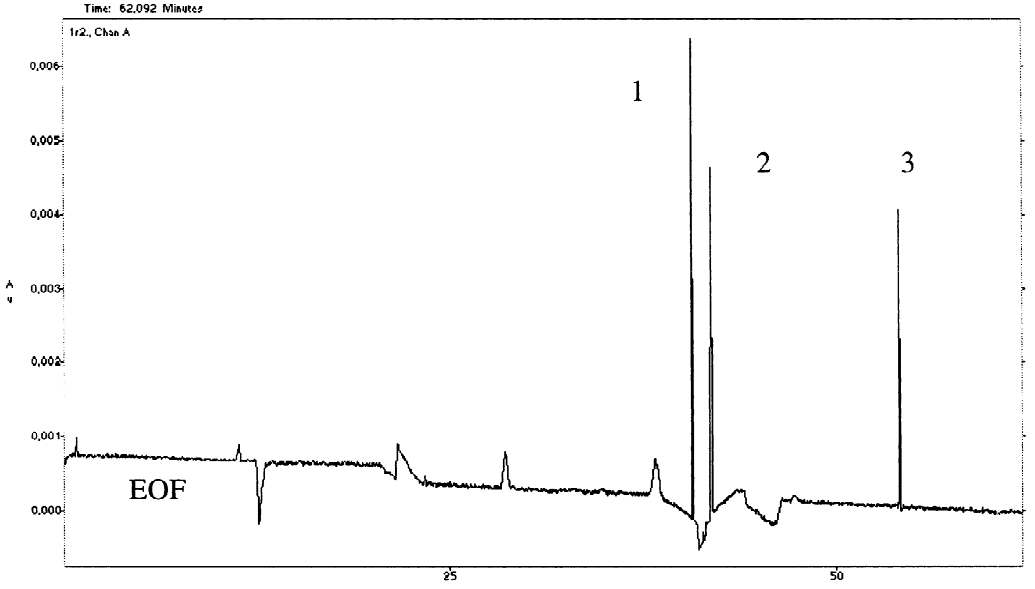

Fig. 6. Separation of small molecule test mixture with the basic (pH 9.5) microemulsion technique. Compounds: (1) nicotine, (2) *N*-acetylprocainamide and (3) procinamide. Sample concentration 50 mg/l. Experimental conditions as in Fig. 2.

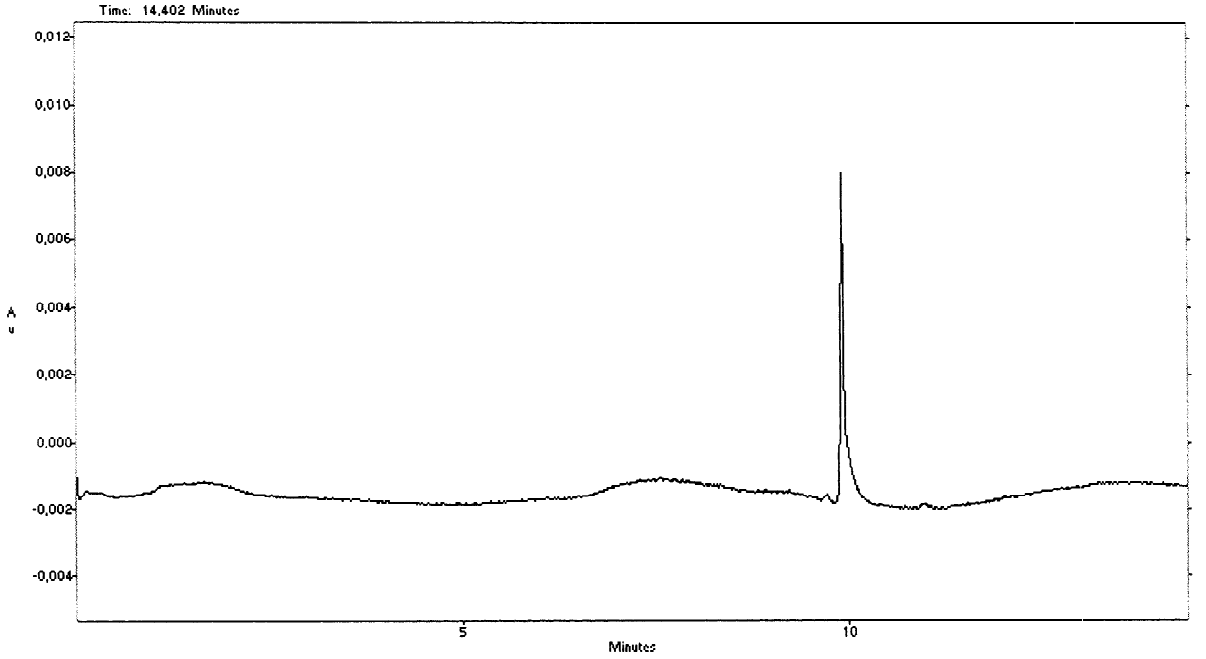

Fig. 7. Migration of cytochrome *c* in basic microemulsion solution. Sample amount: 75 mg/l. Experimental conditions as in Fig. 2.

MEKC, since their migration order did not change. Therefore, in our studies no differences in selectivity between MEKC and MEEKC techniques were noticed on the basis of selectivity. However, the solvent composition affected in sample zone resolution and peak widths.

4. Conclusions

Adjusting the pH with borate to pH 9.5 and using octane as the emulsion former and SDS as the surfactant in 1-butanolic water solution gave good resolution for structurally related polar compounds. The analyses showed that optimisation of the concentrations of SDS and 1-butanol considerably improved the efficiency of the separations. Generally, 4–6 catecholamines and benzodiazepines were separated, but even 14 diuretics were separated from each other in basic microemulsion medium. It was supposed that in the microemulsion solutions proteins formed ion-pairs with the anionic SDS surfactant molecules. Therefore, acid electrolyte solution was used and it was advantageous for analysis of anionic catecholamines and their metabolites too. Although the migration windows for all the drug groups were long, the analytes eluted as sharp zones with high intensities of compounds having low mobilities which would not be possible in MEKC separations.

Acknowledgements

Financial support from the Academy of Finland is gratefully acknowledged.

References

[1] K.D. Altria, B.J. Clark, P.-E. Mahuzier, Chromatographia 52 (2000) 752.
[2] K.D. Altria, J. Chromatogr. 844 (1999) 371.
[3] M.-L. Riekkola, S.K. Wiedmer, I.E. Valkó, H. Sirén, J. Chromatogr. A 792 (1997) 13.
[4] http://www.ceandcec.com/microemu1.htm.
[5] R.L. Boso, M.S. Bellini, I. Miksik, Z. Deyl, J. Chromatogr. A 709 (1995) 11.
[6] X. Fu, J. Lu, A. Zhu, J. Chromatogr. 735 (1996) 353.
[7] S. Terabe, N. Matsubara, Y. Ishihama, Y. Okada, J. Chromatogr. 608 (1992) 23.
[8] M.F. Miola, M.J. Snowden, K.D. Altria, J. Bioanal. Anal. 18 (1998) 785.

[9] S. Pedersen-Bjergaard, C. Gabel-Jensen, S.H. Hansen, J. Chromatogr. A 897 (2000) 375.
[10] I. Erlund, J. Alftan, H. Sirén, K. Ariniemi, A. Aro, J. Chromatogr. B 727 (1999) 179.
[11] K. Vuorensola, H. Sirén, J. Chromatogr. A 895 (2000) 317.
[12] D. Borrey, E. Meyer, W. Lambert, S. Van Calengergh, V. Van Peterghem, A.P. De Leenheer, J. Chromatogr. A 910 (2001) 105.
[13] H. Sirén, U. Karjalainen, J. Chromatogr. A 853 (1999) 527.
[14] H. Bi, S.F. Cooper, M.G. Coté, J. Chromatogr. 582 (1992) 93.
[15] T. Halmou, B. Bourguignon, D.L. Massart, J. Chromatogr. 633 (1993) 43.
[16] M.-L. Riekkola, J.H. Jumppanen, J. Chromatogr. A 735 (1996) 151.
[17] H. Sirén, T. Hiissa, Y. Min, Analyst 125 (2000) 1561.
[18] S.K. Wiedmer, M.-L. Riekkola, Rev. Anal. Chem. 18 (1999) 67.
[19] J.H. Jumppanen, H. Sirén, M.-L. Riekkola, J. Chromatogr. A 652 (1993) 441.
[20] T.-Y. Yen, H. Yan, B.A. Macher, J. Mass Spectrom. 37 (2002) 15.
[21] T.S.K. Paliwal, M. De Frutos, F.E. Regnier, Methods Enzymol. 270 (1996) 133.
[22] H. Kataoka, Y. Ueno, M. Makita, J. Pharm. Biomed. Anal. 10 (1992) 365.
[23] C. Colyer, Cell Biochem. Biophys. 33 (2000) 323.
[24] C.G. Huber, A. Premstaller, G. Kleindienst, J. Chromatogr. A 849 (1999) 175.
[25] D.N. Heigel, High Performance Capillary Electrophoresis, An Introduction, Hewlett-Packard, 1992.
[26] I. Miksik, J. Gabriel, Z. Deyl, J. Chromatogr. A 772 (1997) 297.
[27] C. Gabel-Jensen, S. Honoré-Hansen, S. Pedersen-Bjergaard, Electrophoresis 22 (2001) 1330.
[28] M.F. Miola, M.J. Snowden, K.D. Altria, J. Pharm. Biomed. Anal. 18 (1998) 785.
[29] T. Virta, H. Sirén, unpublished results, 2000.
[30] H. Sirén, K. Vuorensola, J. Microcol. Sep. 13 (3) (2001) 126.
[31] G.-H. Zhou, G.-A. Luo, X.-D. Zhung, J. Chromatogr. A 853 (1999) 277.