ELSEVIER

Journal of Chromatography A, 892 (2000) 171–186

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Background theory and applications of microemulsion electrokinetic chromatography

Kevin D. Altria*

Pharmaceutical Development, Glaxo Wellcome R&D, Park Road, Ware, Herts. SG12 ODP, UK

Abstract

Microemulsion electrokinetic chromatography (MEEKC) is an electrodriven separation technique. Separations are achieved using microemulsions which are nanometre-sized oil droplets suspended in aqueous buffer. The surface tension between the oil and water components is reduced by covered the oil droplet with an anionic surfactant such as sodium dodecyl sulphate and a co-surfactant such as a short-chain alcohol. This review summarises the various microemulsion types and compositions that have been used in MEEKC. The effects of key operating variables such as pH and temperature are also described. The application areas of MEEKC are also described in some detail. MEEKC has been applied to a wide range of water-soluble and insoluble both charged and neutral compounds. Examples are described which include analysis of derivatised sugars, proteins, pesticides and a wide range of pharmaceuticals. At present there are only a limited number of publications describing the use of MEEKC but it is anticipated that this number will increase rapidly in the near future as more awareness of the separation possibilities that MEEKC presents increases.

Keywords: Reviews; Microemulsion electrokinetic chromatography; Pharmaceutical analysis; Buffer composition

Contents

*Tel.: +44-1920-883-616; fax: +44-1920-882-295.
E-mail address: kda8029@ggr.co.uk (K.D. Altria).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00088-1

1. Introduction

Microemulsion electrokinetic capillary chromatography (MEEKC) is an electrodriven separation technique, which offers the possibility of highly efficient separations of both charged and neutral solutes covering a wide range of water solubilities. The technique uses microemulsion buffers to separate solutes based on both their hydrophobicities and electrophoretic mobilities. Microemulsions [1] are solutions containing dispersed nanometre-sized droplets of an immiscible liquid. In particular the microemulsions used in MEEKC are oil droplets dispersed in an aqueous buffer. The oil and water components are totally immiscible and do not mix together as there is a high surface tension between them.

The oil droplets are coated with a surfactant to reduce [2] the surface tension between the two liquid layers which allows the emulsion to form. The surface tension is further lowered, to approach zero, by the addition of a short-chain alcohol such as butan-1-ol which stabilises the microemulsion system. If the microemulsion system was unstable then it will revert to individual layers of oil and water after a short period of time. The diameter of the oil droplets is below 10 nm. The microemulsion is therefore optically transparent as larger droplets scatter white light.

Disintegration of the emulsion system actually represents an activation barrier, as the surface tension of the system is at an energy low. Therefore if the combination of oil, surfactant and co-solvent is appropriate then the microemulsion systems are highly stable and remain intact indefinitely.

Use of a microemulsion containing ionic surfactants allows chromatographic separation to be obtained as solutes can partition between the charged oil droplet and the aqueous buffer phase. Water-insoluble compounds will favour inclusion into the oil droplet rather than into the buffer phase. This situation allows partitioning of the solute between the oil and water phases in a chromatographic fashion. Hydrophobic solutes will reside more frequently in the oil droplet than water-soluble solutes. The separation basis is similar [3] to that involved in micellar electrokinetic chromatography (MEKC) where ionic surfactant monomers group together to form micelles. Solutes chromatographically interact with the micelles to achieve separation. Solutes are more easily able to penetrate the surface of the droplet [3] than the surface of a micelle which is

much more rigid. This ability allows MEEKC to be applied to a wider range of solutes.

Sodium dodecyl sulphate (SDS) is the most widely used emulsifier surfactant in MEEKC. The oil droplet is coated with SDS surfactant molecules making the droplet negatively charged. The C_{12} alkyl chain of the surfactant penetrates into the oil droplet whilst the negatively charged hydrophilic sulphate groups resides in the surrounding aqueous phase. Charge repulsion of the negatively charged sulphate group on the SDS prevents highly efficient packing and prevents formation of an emulsion as the surface tension cannot be sufficiently reduced. A co-surfactant, usually a medium-chain-length alkyl alcohol such as butan-1-ol, is therefore essential in the formation and improved stability of the microemulsion. The co-surfactant bridges the oil and water interface and further reduces the surface tension of the system to zero. Fig. 1 provides a schematic of the emulsion droplet showing the short-chain alcohol, SDS, the octane droplet and the sodium ions surrounding the droplet.

The microemulsion is a dynamic entity and it has a lifespan [4] in the microsecond range. The emulsion droplets exist in a variety of shapes with an average that is spherical. The range of droplet shapes that an individual emulsion system contains has been measured [3] and is known as the polydisperity. A highly ordered microemulsion system has a low polydisperity and highly spherical droplets. The surfactant and co-surfactant act together to reduce the surface tension between the two liquid phases to zero. The presence of the co-surfactant helps the droplets to pack more effectively and create a more stable environment.

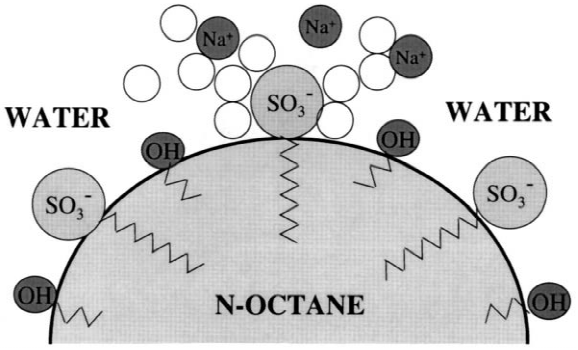


Fig. 1. Schematic of surfactant coated oil droplet.

High pH buffers such as borate or phosphate are generally used in MEEKC. These buffers generate a high electroosmotic flow (EOF) when a voltage is applied across a capillary filled with the buffer. This flow is relatively rapid and is towards the cathode situated near the detector. The surfactant-coated oil droplets are negatively charged and therefore attempt to migrate towards the anode when the voltage is applied. However, the EOF is sufficiently strong to eventually sweep the oil droplets through the detector to the cathode. Highly water-soluble neutral solutes such as methanol will reside predominantly in the aqueous phase and will be swept rapidly to the detector by the EOF giving a solvent front (t_0) measurement.

Conversely a highly water insoluble solute such as dodecylbenzene will predominately favour partitioning into the negatively charged droplet and will be strongly retained with an infinitely high capacity factor. If a moderately soluble solute has a capacity factor (k') of 1 then it spends equal amounts of time in both the aqueous phase and the oil droplet. The MEEKC migration time, or capacity factor, of a neutral solute can be directly related to the solubility (hydrophobicity) of the solute. MEEKC has been used [5–7] to assess compound solubility with good cross-correlation to other techniques used to measure solubility.

The retention time, t_r for a neutral species is always between t_0 and t_{ME}:

$$t_r = \left(\frac{1 + k'}{1 + \frac{t_0}{t_{ME}} k'} \right) \cdot t_0$$

where t_0 is the time required for an unretained substance such as methanol to travel through the capillary (from injection point to detection window), t_{ME} is the time required for a microemulsion droplet to traverse the capillary – this has been measured from the migration time of a highly retained compound such as dodecylbenzene.

If a solute is ionised then it will electrophoretically migrate according to its size and number of charges when the voltage is applied. Repulsion from the negatively charged droplet will occur if the solute is

also negatively charged. Conversely if the solute is positively charged it may have ion-pair type interactions with the negatively charged droplet. The migration times obtained in MEEKC for ionised solutes reflects [7] a combination of both the electrophoretic and chromatographic behaviour of the solute ion.

The separation principle in MEEKC (Fig. 2) is similar to that occurring in micellar electrokinetic chromatography (MEKC). Aqueous high pH buffer solutions containing relatively high levels of SDS are generally used in MEKC. At SDS concentrations above 10 m*M* the SDS molecules group together to form negatively charged micelles. Solutes can partition with the negatively charged micelles which attempt to migrate against the EOF. Water-insoluble solutes favour inclusion into the micelle and therefore have long migration times. MEKC is a well-established separation technique that has been studied extensively and it is known that selectivity in MEKC can be altered by a variety of factors [8] including variation of the surfactant type and concentration, and the use of buffer additives such as organic solvents, urea, cyclodextrins and ion-pair reagents. Operating temperature, pH and choice of sample dissolving solvent also influence selectivity. A microemulsion system is more complex than a micellar solution and there are more operating variables in MEEKC such as the concentration and choice of the oil and the co-surfactant.

The options available in method development in MEEKC will be described which include type and concentration of surfactant, buffer pH and the type of oil. Selectivity can also be affected by organic solvents, type of co-solvent, addition of cyclodextrins ion-pair reagents and the manufacture process for the microemulsion. These options and their particular ranges will be described in this paper.

MEEKC is a relatively recent technique and it has not been widely applied to a range of application types. However, there are currently sufficient applications to demonstrate the widespread potential uses of MEEKC. The reported application range will be summarised to provide an appreciation of the separation possibilities that MEEKC may offer.

Overall it is concluded that MEEKC can offer the possibility of highly efficient separations of a range of solute types. The technique can be equally applied to water-soluble and insoluble compounds and to charged or neutral solutes. It is predicted that use of MEEKC will increase rapidly as awareness of these possibilities increases.

2. Method development options

The complexity of the composition of the microemulsion and the MEEKC separation process allows a great many manipulations to be made during method development in order to achieve a particularly difficult resolution. In specific the choice

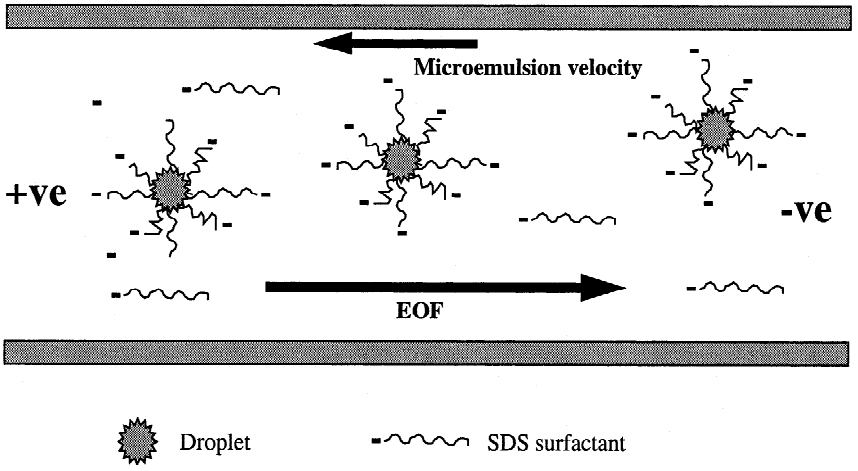


Fig. 2. Principles of MEEKC.

of ionic surfactant can affect the charge and size of the droplet and the magnitude and direction of the EOF. The pH of the buffer also affects the magnitude of the EOF and the charge on ionic solutes. The choice of the oil will effect the solute partitioning coefficient and has an effect on the selectivity. Selectivity can also be altered by factors such as the type and concentration of co-surfactant and the buffer. Selectivity can be further manipulated by the addition of organic solvents, cyclodextrins, urea and ion-pair reagents. Factors such as the operating temperature and the sample diluent also affect selectivity.

2.1. Surfactant type and concentration

The choice of surfactant has a marked effect on the separation achieved in MEEKC as it affects the oil droplet charge and size, the level and direction of the EOF, and the level of any ion-pairing with charged solutes. SDS is an anionic surfactant with a C_{12} alkyl chain, which penetrates into the oil droplet. Anionic bile salt surfactant such as sodium cholate has also been used [9] to generate negatively charged droplets. Use of bile salt microemulsions gave different [9] selectivity compared to when SDS was used to make the microemulsions.

Cationic surfactants such as cetyltrimethylammonium bromide (CTAB) have also been used [10] in MEEKC. CTAB is a positively charged surfactant and has a C_{16} alkyl chain which penetrates into the oil droplet. CTAB produces positively charged droplets and also generates a positively charged surfactant bilayer on the capillary wall which reverses the EOF direction. A negative polarity voltage is therefore used when working with CTAB microemulsions. In particular CTAB-based microemulsions have been used [5] to eliminate ion-pair interactions that cationic solutes have with anionic SDS microemulsion droplets.

Neutral surfactants such as Tritron X-100 can be used [11] to make microemulsions but these are not useful for separating neutral solutes as the neutral droplets migrate at the same speed as the EOF and the neutral solutes.

Higher concentrations of the surfactant increases [12] the capacity factor of neutral solutes as it increases the charge density [13] on the oil droplet. If a mixture of charged and neutral solutes are employed then altering the surfactant concentration will have an effect on peak migration order. Increasing the surfactant concentration also increases the ionic strength of the buffer which reduces the EOF level and increases analysis time.

Increased chain length of the surfactant stabilises the microemulsion [2] as it reduces the polydispersity of the emulsion droplet size.

Higher levels of surfactant reduce surface tension to a greater extent which generates more stable microemulsions. Typically SDS concentrations in the region of 110 m*M* (3%, w/w) are used which produces highly stable microemulsions with shelf lives exceeding several months. SDS concentrations as high as 6.5% (w/w) have been used. Terabe et al. [3] reported instability of the microemulsions using only 1.6% (w/w) SDS. Ishihama et al. [5] also reported poor repeatability using 1.4% SDS. In a previous work [14] we observed that microemulsions produced with 2% (w/w) or less SDS content disintegrated after only a few hours.

2.2. The effect of pH

The pH of the buffer has a pronounced effect on the separation selectivity as it affects both solute ionisation and the level of EOF generated. Typically buffers in the region of pH 7–9 have been used in MEEKC which generate relatively high EOF velocities. Ionic compounds are generally ionised at these pH values. Basic drugs typically remain protonated until high pH values such as pH 12–13 are employed. Acidic drugs typically have pK_a values in the region of pH 3–6 and are therefore ionised at the pH values typically used in MEEKC. Ionised solutes will have different migration properties compared to neutral compounds. Positively charged basic drugs will have both partitioning and ion-pair interactions with the negatively charged oil droplet. The basic drugs will also have an electrophoretic mobility which reduces their MEEKC migration times. Conversely acidic solutes will have a negative electrophoretic mobility, will partition into the droplet but will be charge repelled from the negative droplet.

Extremes of pH have been used in MEEKC to specifically suppress solute ionisation. For example pH 1.2 buffer has been used [7,15] to prevent the

ionisation of acids. This low pH also eliminates EOF. A negative voltage is therefore employed to attract the droplets towards the detector which results in the most highly retained solutes being detected first which is opposite to that observed at normal pH values.

A pH 12 buffer has been used [7] to eliminate the ionisation of basic compounds. These pH extremes were used when MEEKC was used [7] to measure the solubility of ionic compounds based on their MEEKC migration times. To measure the solubility accurately it was important that the solute is uncharged. High-pH carbonate buffers have also been used [15] in place of the standard borate or phosphate.

Buffers with high pH values are normally used as these give faster EOF rates. This is not the case when using cationic surfactants, which adsorb onto the capillary wall and form a positively charged bilayer. At high pH values hydroxide ions adsorb onto the positively charged wall and reduce the flow-rate.

2.3. Oil type

Generally octane [16,17] or heptane [18,19] has been used to generate the oil droplet. Heptane may be preferred as the odd numbered alkanes have [20] lower toxicity than the even numbered alkanes. However, octane has been reported [21] to give more repeatable microemulsions than heptane. Hexane, heptane and octane have been shown [21] to give similar selectivity and migration times for separation of a range of neutral compounds.

A range of other water-immiscible liquids have been employed [10] including diethyl ether, cyclohexane, chloroform, methylene chloride and amyl alcohol. Other oils used in MEEKC include butyl chloride [21], ethyl acetate [22], octan-1-ol [23] and hexan-1-ol [24].

A chiral oil (2*R*,3*R*)-di-*n*-butyl tartrate has been used [25] in MEEKC to achieve chiral separation of racemic ephedrine.

2.4. Addition of organic solvents

In MEKC highly water insoluble solutes partition strongly into the micelles and are therefore highly retained with poor resolution of mixtures. In these instances organic solvent are often added into MEKC buffers to reduce retention and improve resolution. Standard solvents used are acetonitrile, methanol and isopropanol. This approach has also been used in MEEKC. Li et al. reported [22] the addition of 15% (v/v) acetonitrile to a MEEKC buffer to improve resolution of natural product components. The addition of 8% (v/v) methanol was shown [5] to give shorter migration times with the same selectivity.

The levels of solvent that can be added to MEKC buffers is generally limited to a maximum such as 30% (v/v). At levels greater than this the micelle are disrupted and selectivity is lost. It was found that there were also limits to the maximum solvent contents that could be used in MEEKC. When these limits were exceeded the microemulsion buffers disintegrated into a cloudy two-phase system, which could not be used for separation.

2.5. Co-surfactant type and concentration

Butan-1-ol is the most frequently employed co-surfactant. Studies have shown that the separation selectivity is unaltered [13] by variation of the butan-1-ol concentration. Higher butan-1-ol concentrations reduce [12] migration times for water-insoluble solute but do not alter the capacity factors. The migration times are altered with varying co-surfactant concentration as it affects the solution viscosity which in turn affects the EOF rate. The size of the oil droplet increases [26] with increased co-surfactant concentration which will affect the charge density on the droplet. Variation of co-surfactant concentration over a wide range affected [27] the migration times of hop bitter acids.

2.6. Buffer type and concentration

Generally MEEKC has been performed [17,28] using low-ionic-strength (5–10 m*M*) borate or phosphate buffers. These generate relatively low currents and a reasonably fast EOF. Higher buffer concentrations suppress the EOF and generate high currents which may limit the level of voltage that can be applied. MEEKC has been operated with buffers as high as 100 m*M* borate.

2.7. Sample diluent

Poor separation is obtained if the sample is not dissolved in the microemulsion. This is due to disruption of the microemulsion environment inside the capillary that is adjacent to the sample injection plug. The sample diluent can cause the microemulsion to disintegrate back to unmixed oil and water layers. This is seen as both reduced migration times and capacity factors, and peak tailing. This effect is also observed in MEKC [29] especially when the sample is dissolved in organic solvents as this causes the micelles near the sample zone to collapse.

Injection of samples dissolved in methanol gave [17] extremely poor separation compared to when the sample was dissolved in microemulsion solution – especially for longer injection times.

2.8. Microemulsion preparation procedure

There are a number of procedures that can be adopted when preparing the microemulsions. The most common approach [17] is to weigh the ingredients together which produces a cloudy suspension. This suspension is then sonicated for 30 min to generate an optically clear solution. An alternative approach [10] is to vortex mix the aqueous buffer/surfactant solution and oil together. The butan-1-ol is then added dropwise until an optically clear solution is spontaneously generated when the surface tension in the solution sufficiently approaches zero. It has been seen [30] that evaporation losses of heptane were reduced if it was added to the microemulsion ingredients after the butan-1-ol. An alternative means [31] of producing the microemulsion is to mix the surfactant, co-surfactant and oil together and then add the buffer containing additional surfactant to the stirred mixture until a clear solution is obtained. It has also been reported that it is necessary to sonicate the microemulsion buffer for an extensive period [5] to prevent the microemulsion becoming turbid upon standing at room temperature but this would be dependent on the microemulsion composition.

2.9. Temperature effects

To date, there has been no evaluation of the temperature effects in MEEKC. However preliminary work in our laboratory has shown that they are significant and the temperature should be well controlled. The temperature affects solute solubility, which is related to the partitioning coefficient. The electrophoretic mobility of an ion is also affected by temperature by 2% per °C. The selectivity of a test mixture of neutral solutes is unaffected by temperature. However, there are selectivity variations when the test solution contains both charged and neutral solutes as the temperature has disproportionate effects on the charged and neutral species.

3. Applications

3.1. Solubility (hydrophobicity) assessments

The solubility (log P) of a neutral solute can be directly assessed from migration time data obtained in MEEKC. This is the most frequently reported application of MEEKC where solubility data has been obtained for neutral [6,10], anionic [7,32] and cationic [7,33] solutes. Typically the migration times of a number of solutes with known log P are determined to generate calibration graph or migration index. Fig. 3a shows separation of eight neutral solutes using SDS–octane–butan-1-ol microemulsion. Fig. 3b shows the migration time data for these solutes plotted against their corresponding log P values. The migration index (MI) is calculated [5] by:

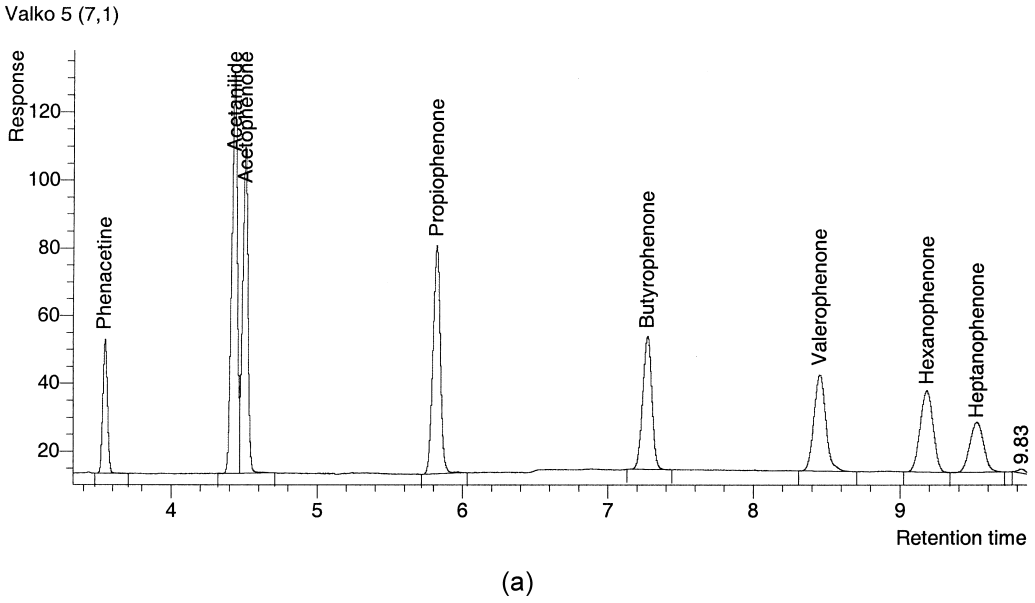
$$\mathrm{MI} = c \log k' + d$$

where c and d are the slope and intercept of the calibration line, respectively.

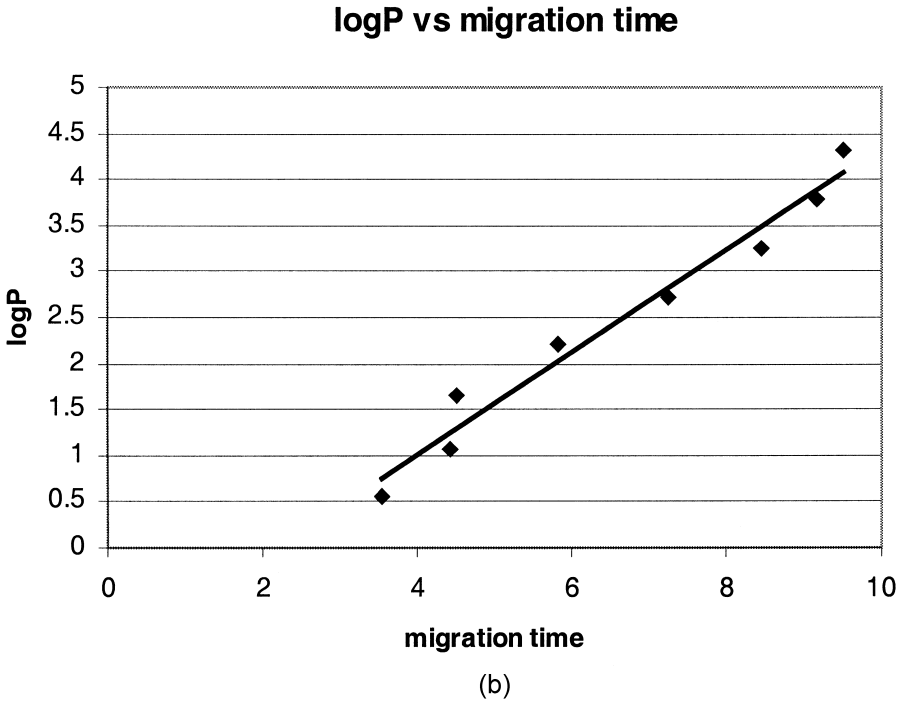
3.2. Derivatised sugars

Generally carbohydrates are neutral and non-chromophoric and it is generally necessary to derivatise them prior to analysis. Further details can be found in the recent book by Paulus and Klockow-Beck [34] which describes the analysis of carbohydrates by capillary electrophoresis (CE). These analytical methods have included CE using simple buffers and MEKC has been applied to separate sugar derivatives.

MEEKC and MEKC have both been applied [23]



(a)



(b)

Fig. 3. Use of MEEKC to determine partition coefficients. (a) Separation of a range of phenones by MEEKC. Separation conditions: 0.81% (w/w) octane, 6.61% (w/w) butan-1-ol, 3.31% (w/w) sodium dodecyl sulphate and 89.27% (w/w) 10 m*M* sodium tetraborate buffer, 15 kV, 30 cm×50 μm I.D. capillary (detection window at 22 cm), 40°C, 200 nm. (b) Plot of MEEKC migration times versus log *P* data for a range of phenones.

to the separation of highly insoluble diphenyl hydrazine derivatives. A 10-component carbohydrate test mixture was resolved by MEEKC using a SDS–octane–butan-1-ol microemulsion system. However, the borate–SDS MEKC system only permitted resolution of the test mixture into three multi-component peaks. The MEEKC method was then successfully used to profile the carbohydrate content in Daeolalea Quereina cultivates.

3.3. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are generally difficult to analyse by CE as they are neutral and possess low water solubilities. Very specific MEKC methods [35] involving use of high levels of organic additives or cyclodextrins [36] have been successful in the analyses of PAHs. Test mixtures of PAHs have been successfully resolved many times [37] in capillary electrochromatography (CEC) which is ideally suited to this analysis type. MEEKC has been used to separate a range of simple low-molecular-mass neutral aromatics [38] including naphthalene using a SDS–heptane–butan-1-ol microemulsion. Simple aromatic solutes such as naphthol and toluene have also [3] been separated using SDS–heptane–butan-1-ol microemulsions.

Attempts to separate PAHs using SDS–octane–butan-1-ol were unsuccessful as the highly water-insoluble solutes were precipitated in the buffer or were highly retained with no resolution. Methanol was therefore added to the buffer to increase solute solubility and to reduce solute retention. The addition of organic solvents has been used once before in MEEKC for the separation of water-insoluble natural products [22] where 15% acetonitrile was added to reduce retention and improve resolution. Excessive quantities of acetonitrile caused [22] loss of selectivity.

Fig. 4 shows separation of five PAHs using MEEKC containing 10% ethanol. These compounds

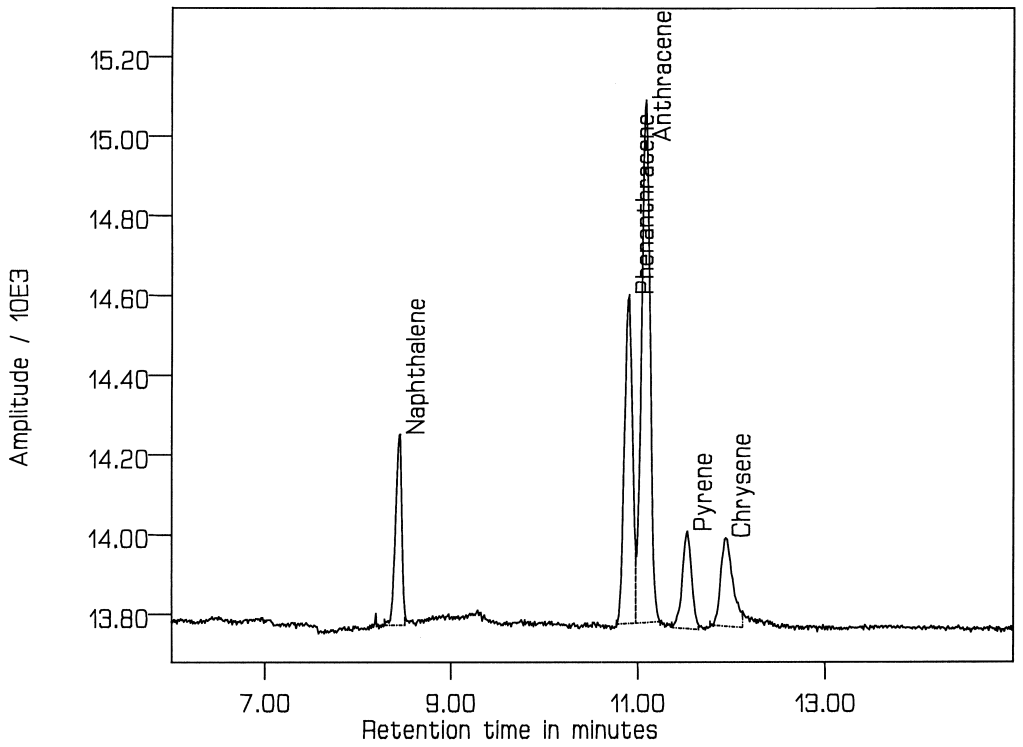


Fig. 4. Separation of a range of polycyclic aromatic hydrocarbons using a MEEKC buffer containing ethanol. Separation conditions as in Fig. 3a except 90% (v/v) [0.81% (w/w) octane, 6.61% (w/w) butan-1-ol, 3.31% (w/w) sodium dodecyl sulphate and 89.27% (w/w) 10 m*M* sodium tetraborate buffer], 10% (v/v) ethanol.

were initially dissolved in methanol and then diluted with the MEEKC buffer.

Fluorescene detection has been used [15] to monitor separation of four napthalene derivatives using a SDS–heptane–butan-1-ol microemulsion system.

3.4. Proteins

MEEKC has been used to separate a range of proteins [39]. The proteins were separated, largely based on their hydrophobicities, using an SDS–heptane–butan-1-ol microemulsion in 2.5 m*M* borate buffer. Resolution of the separated proteins was strongly affected by the SDS concentration with maximum resolution obtained at 120 m*M* SDS. The resolution obtained for ribonuclease A, carbonic anhydrase II, β-lactoglobulin A and myoglobulin by MEEKC was better than conventional CE using a borate buffer or MEKC. Proteins are generally too large to partition into a micelle but can partition into the microemulsion droplet which has a larger volume. The MEEKC method resolved both basic and acidic proteins and was applied to the analysis of a range of injection formulations containing various protein mixtures.

3.5. Hop bitter acids

Hop bitter acids are present in the hops used to manufacture beer. The levels and composition of these acids affect the quality of the hops and is therefore tested before the hops are used in beer manufacture. MEEKC has been shown [27,40] to give accurate and precise data for this analysis. Resolution of the six major hop acids was achieved within 10 min with separation efficiencies in the order of 280–480 000 theoretical plates. Resolution and separation efficiency was shown [27] to be superior to that obtained by MEKC and the analysis time was shown [40] to be shorter than for high-performance liquid chromatography (HPLC) (typically 45–60 min). The hop acids were detected at 214 mm in MEEKC to give acceptable sensitivity. Table 1 shows that MEEKC and HPLC gave equivalent quantitative data for the hop acid ratio composition of various samples.

Table 1
Analysis of hop bitter acids using MEEKC[a]

Hop cultivar	Cohurmulone ratio		Colupulone ratio	
	HPLC	MEEKC	HPLC	MEEKC
Saaz I	24.6	24.1	42.8	44.0
Saaz II	24.3	25.7	43.5	42.7
Saaz III	25.2	24.4	42.8	43.1
Nugget I	35.8	35.8	60.5	60.9
Nugget II	34.6	37.3	60.0	59.7
Nugget III	35.2	36.5	60.4	59.2
Wye Target I	40.6	41.6	60.2	60.7
Wye Target II	39.5	41.9	60.3	59.7
Wye Target III	39.8	41.2	61.2	60.0

[a] Reproduced with permission from Ref. [27].

3.6. Agrochemicals

CE has been successfully applied [41] to a range of agrochemical determinations. The water-insoluble neutral compounds such as phenylurea herbicides require chromatographic based methods whilst water-soluble acidic compounds such as chlorinated acids and phenols can be analysed [42] using high-pH CE buffers.

Resolution of six phenylurea herbicides and chlorsulphuron was achieved [13] using a SDS–octane–butan-1-ol microemulsion system. The highly insoluble herbicides were dissolved in *N*,*N*-dimethylformamide. The effect on the resolution was assessed for a range of operating parameters such as voltage, and the concentration of the butan-1-ol, SDS and oil.

3.7. Vitamins

Vitamins are classified into water- or fat-soluble species. The water-soluble acidic vitamins such as nicotinic acid and vitamin C possess an acidic function and these can be analysed using CE with high-pH borate or phosphate buffers. However, the fat-soluble vitamins such as vitamins A and E are neutral, have poor water-solubility, and require use of a chromatography-based method. MEEKC has been shown [17] to be useful for the simultaneous determination of water- and fat-soluble vitamins.

An extensive study by Boso et al. [10] investigated the selectivity obtained for a vitamin test mixture when using a range of various oil and surfactant types and concentrations. A complex

multi-vitamin pharmaceutical formulation was analysed [17] using an SDS–octane–butan-1-ol microemulsion.

A vitamin formulation containing both water-soluble and insoluble vitamins has been resolved (Fig. 5) using MEEKC. A 1-ml volume of the liquid formulation was diluted to 5 ml with the microemulsion buffer and directly injected.

3.8. Ketones and β-diketones

Test mixtures of various ketones such as acetylacetone, benzoylacetone, acetophenone and benzyoyltrifluoroacetone, were separated [10] using a high-pH carbonate buffer containing SDS, heptane and butan-1-ol. The MEEKC method was shown to give both improved resolution and analyte solubility range than a micellar method. Detection of non-chromophoric ketones was achieved [43] by indirect fluorescent detection following the addition of naphthalene to the microemulsion buffer.

3.9. Pharmaceuticals

The analysis of pharmaceuticals is the most frequent application of CE [44] and MEEKC has been used to separate and quantify a range of pharmaceutical classes.

3.9.1. Analgesics/cold medicine ingredients

A test mixture of seven cold medicines were separated [3] using a heptane–SDS–butan-1-ol microemulsion. Separation efficiencies obtained in MEEKC [3] were higher than those obtained in MEKC for the same test mixture. Antipyrene analgesics were separated [21] using either octane, heptane or 1-butyl chloride as the core oil. Octane was shown to give the best migration time precision in a short precision study.

The active components and parahydroxybenzoate preservatives in a cold medicine formulation have been separated and quantified using a MEEKC method. The liquid formulation was diluted with the

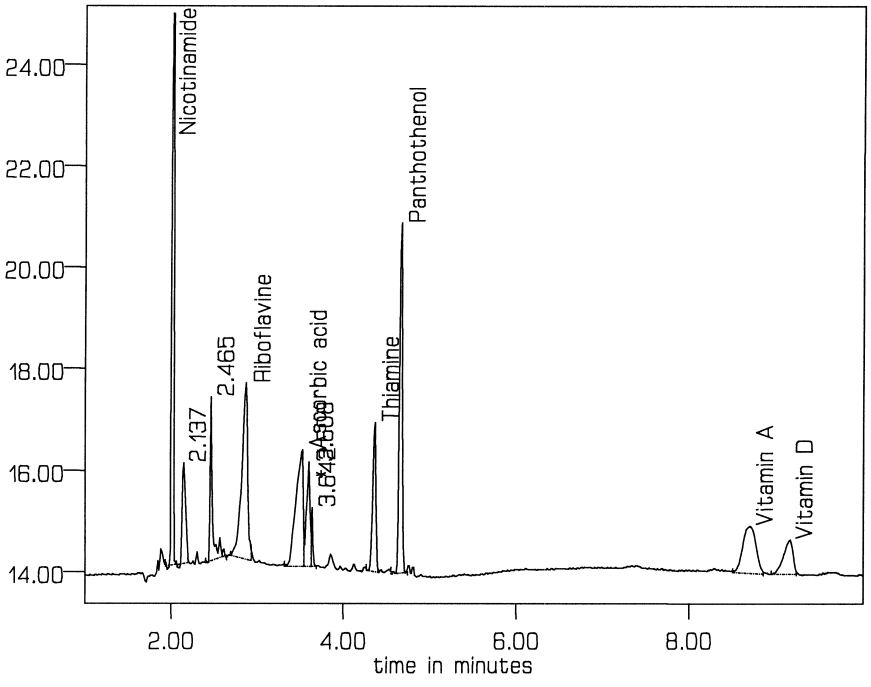


Fig. 5. Separation of a range of water-soluble and insoluble vitamins in a liquid formulation. Separation conditions as in Fig. 3a. Reproduced with permission from Ref. [17].

Table 2
Comparison of the steroid content by HPLC and MEEKC[a]

Sample	Activity	
	HPLC	MEEKC
1	0.00	0.00
2	0.42	0.44
3	0.96	0.94

[a] Reproduced with permission from Ref. [24].

microemulsion buffer [17] and directly injected into the capillary.

3.9.2. Steroids

Steroids are often difficult to analyse by CE as they are generally neutral and water-insoluble. CEC has been shown [37] to be useful for steroid analysis. A range of different microemulsion compositions were compared [24] for the separation of 10 steroids. Six different oils were assessed including hexane, cyclohexane, hexanol and octanol. Hexanol was considered to be optimal. Various surfactants were assessed with SDS being the optimal. The MEEKC method was applied [24] to the measurement of 11-β-hydroxysteroid dehydrogenase activity in rat intestine. Table 2 shows comparable results to those obtained by a HPLC method.

3.9.3. Basic drugs

Basic drugs can interact with the surface silanols on the stationary phases used in HPLC which can lead to tailing and loss of separation efficiencies. Efficient separation of basic drugs by CE using low-pH buffers is possible. Highly efficient MEEKC separations of a range of water-soluble and insoluble basic drugs including terbutaline, bupivacaine and amitryptline have [17] been demonstrated (Fig. 6) with no evidence of peak tailing. The separation achieved in MEEKC is based on solute partitioning into the droplet, ion-pair interaction with the surface of the droplet and electrophoretic migration of the positively charged compound. To eliminate the ion-pair and migration aspects it is possible [7] to employ high-pH (pH 13) microemulsions where the basic drug will be neutral and will separate solely by partitioning with the droplet.

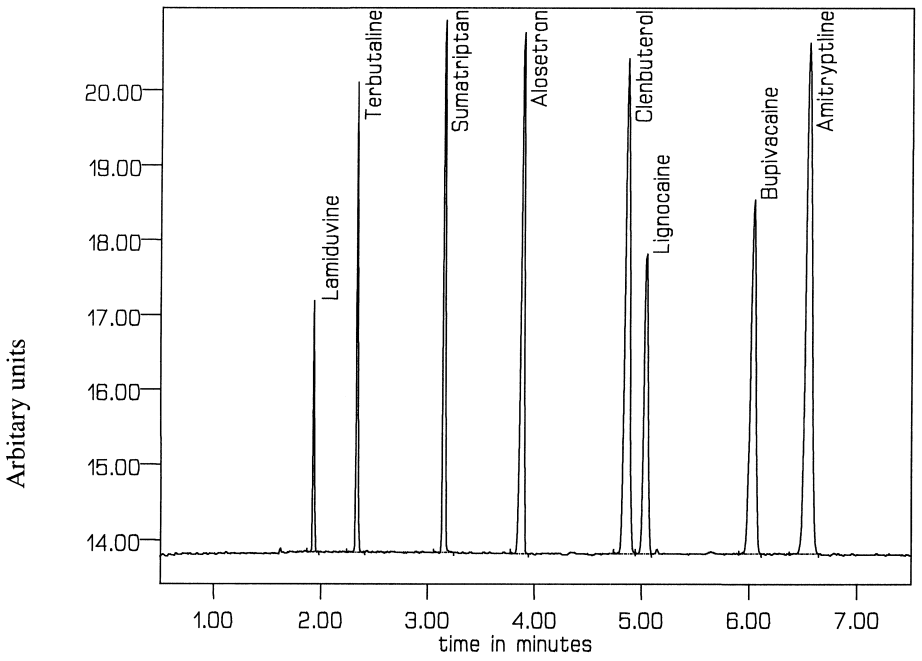


Fig. 6. Separation of a range of water-soluble and insoluble basic drugs. Separation conditions as in Fig. 3a. Reproduced with permission from Ref. [17].

3.9.4. Acidic drugs

A range of water-soluble and insoluble acidic drugs has been resolved [17] by a high-pH MEEKC method. These included a range of related cephalosporins, acetylsalicylic acid and insoluble drugs such as ibuprofen, indomethacin and troglitazone. The method was used to quantify levels of troglitazone in a tablet formulation. An assay result of 199.4 mg/tablet was obtained compared to the label claim of 200 mg/tablet.

3.9.5. Pharmaceutical excipients

A single set of MEEKC operating conditions have been shown [17] to be useful for the analysis of sweeteners such as aspartame and saccharin, preservatives such as parahydroxybenzoates and various dyestuffs.

3.9.6. Cardiac glycosides

This class of natural products compounds includes digoxin which is extracted from foxglove plants. The compounds are toxic at high doses but at controlled levels can be used to regulate heart rate. The compounds are highly insoluble, neutral and possess limited chromophores. MEEKC has been used to separate [30] a range of related glycoside with detection at low UV wavelength as the compounds possess limited UV activity.

3.9.7. Drug characterisation

Microemulsions are employed as pharmaceutical formulations [1] especially for insoluble compounds. CE has been used [31] to measure the partitioning of compounds into various microemulsion systems. A non-microemulsion CE method was used to quantify levels of drugs partitioned into various microemulsion systems. A similar study was performed [31] to assess the partitioning behaviour of a range of cephalosporins.

3.10. Natural product analysis

CE has been applied to the analysis [45] of highly complex natural product extracts. These extracts often contain components having a wide range of polarities and solubilities. Gradient HPLC conditions are generally required to achieve the resolution and solute range required.

MEEKC has been applied to the separation and identification of the active components in Rheum plant extracts. The highly insoluble components were extracted into chloroform or ethanol. A microemulsion comprising ethyl acetate–SDS–butan-1-ol was used for separation. Resolution was further increased by the addition of acetonitrile. The method was used to quantify components in plant extracts. Recovery data in the range of 95–104% were reported. Acceptable linearity data of greater than 0.99 was demonstrated for detector response for the five active components.

Fig. 7 shows the complex separation obtained for a methanolic plant extract using a SDS–octane–butan-1-ol microemulsion system with detection at 200 nm.

3.11. Chiral separation

To date, there has been only one report [25] of chiral resolution employing MEEKC. This paper showed resolution of ephedrine enantiomers using a microemulsion buffer containing a chiral oil. This oil, (2*R*,3*R*)-di-*n*-butyl tartrate, has an enantioselective interaction with the different ephedrine enantiomers. The (2*S*,2*R*)-enantiomer was more soluble in the oil and was therefore more highly retained and migrated later. The microemulsion buffer used was 97.7% (w/w) aqueous Tris buffer, 0.6% (w/w) SDS, 1.2% (w/w) butan-1-ol and 0.5% (w/w) (2*R*,3*R*)-di-*n*-butyl tartrate.

3.12. Urine

CE has been widely used for analysis of biosamples [46]. In particular [46] the use of MEKC has been shown to be useful to reduce the need for extensive sample pretreatment as interfering components such as proteins are solubilised by the micelles and do not mask the peaks of interest which can allow direct injection of complex samples such as plasma. MEEKC has been shown [28] to be useful for bioanalysis. An untreated urine sample was directly injected using a MEEKC buffer system and a complex, highly efficient separation was obtained.

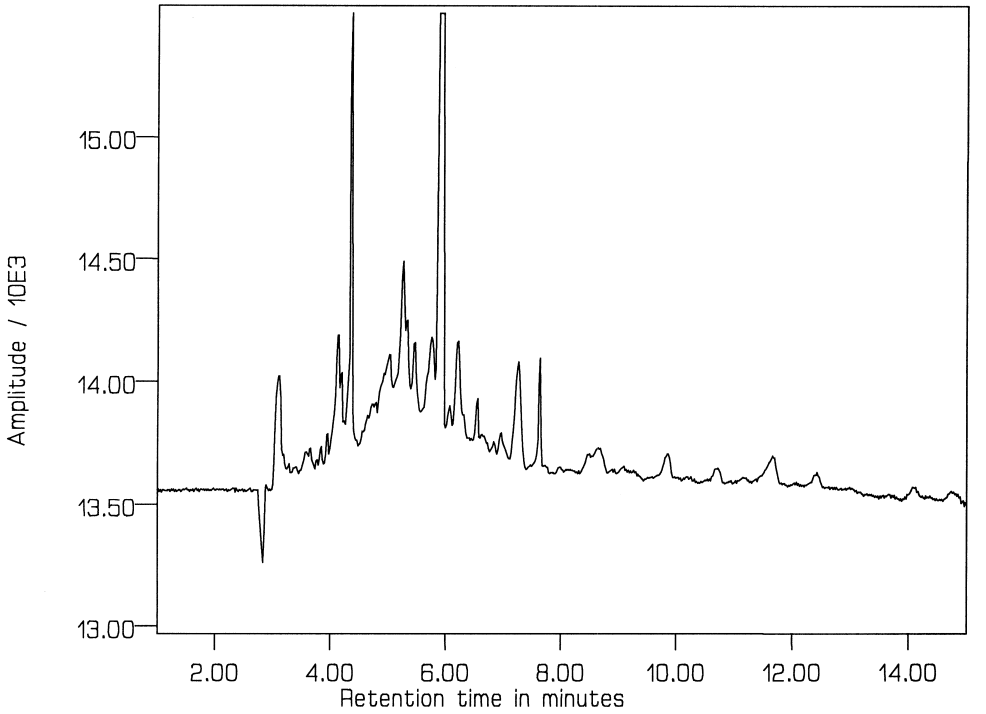


Fig. 7. Analysis of a methanolic plant extract using MEEKC. Separation conditions as in Fig. 3a. Reproduced with permission from Ref. [28].

3.13. Dyes

Conventional CE methods have been applied [47] to the analysis of dyes. In particular acidic dyes have been separated using high buffer systems. MEEKC has also been used [28] to analyse the dye components in fountain pen ink. The sample was diluted with MEEKC buffer and was resolved into a broad range of efficient, well-separated water-soluble and insoluble components.

3.14. Acidic compounds

Acidic compounds have been analysed successfully by CE [48] and CEC [49]. These compounds have also been analysed [28] by MEEKC using high-pH SDS–octane–butan-1-ol microemulsions. The acids are ionised at this pH and separate due to both their electrophoretic migration against the EOF and their partitioning with the oil droplet. This dual separation process creates a significantly different selectivity to that obtained in CE. The separation of acids is difficult in CEC and is performed at low pH where the acids are unionised.

Aromatic acids such as benzoic acid, naphthalene dicarboxylic acid and salicylic acid have been separated [28]. A range of amino acids and amino acid esters has also been resolved [17] by MEEKC. The esters are neutral and would not have been separated using CE, which highlights the utility of MEEKC.

3.15. Fatty acid esters

These compounds are difficult to analyse by conventional CE as they have poor aqueous solubility and low UV activity. It is possible [23] to separate them by CE using a high pH buffer coupled with indirect UV detection. Derivatives of fatty acids such as phenacyl esters are often prepared to give enhanced UV detection possibilities. A MEEKC method with a cholate–heptane–butan-1-ol–borate microemulsion and detection at 243 nm has been

used to separate fatty acid esters ranging from C_2–C_{20}. This compared favourably with a cholate-micellar method, which was only able to separate acids up to C_8.

4. Conclusions

MEEKC is a relatively recent CE technique, which offers the possibility of separating a wide range of compound types. The majority of MEEKC reports have concentrated on the separation of poorly soluble neutral species such as fat-soluble vitamins, steroids and fatty acids. Recent reports have shown that MEEKC can be equally applied to water-soluble charged solutes such as basic drugs, aromatic acids and proteins.

MEEKC methods can be quantitative and results have been cross-validated with other techniques. If the microemulsion composition is correct then good buffer repeatability and stability can be achieved. The selectivity of a separation can be manipulated by a variety of parameters including surfactant concentration and type, pH and oil type. These factors and others have not been fully evaluated so there is scope for considerable academic research.

The operating benefits of MEEKC are similar to all CE techniques in terms of cost and time savings. In particular MEEKC offers the possibility of resolving complex mixtures of solutes having very wide ranges of polarity and solubility. The major limitation may be that the microemulsion buffer is not MS compatible.

MEEKC was introduced [15] in 1991 by Watarai and there are now a total of some 30+ papers in the area of MEEKC. It is expected that this number will greatly expand in the near future as more application chemists and research scientists become aware of the separation possibilities that MEEKC offers.

Acknowledgements

Thanks are extended to Dr Klara Valko of Glaxo-Wellcome for kindly supplying the log *P* data in Fig. 3.

References

[1] P. Kumar, K.L. Mittal, Handbook of Microemulsion Science and Technology, Marcel Dekker, New York, 1999.
[2] F. Sicoli, D. Langevin, J. Phys. Chem. 99 (1995) 14819.
[3] S. Terabe, N. Matsubara, Y. Ishihama, Y. Okada, J. Chromatogr. 608 (1992) 23.
[4] A. Berthod, M. De Carvalho, Anal. Chem. 64 (1992) 2267.
[5] Y. Ishihama, Y. Oda, K. Uchikawa, N. Asakawa, Anal. Chem. 67 (1995) 1588.
[6] M.H. Abraham, C. Treiner, M. Roses, C. Rafols, Y. Ishihama, J. Chromatogr. A 752 (1996) 243.
[7] S.J. Gluck, M.H. Benko, R.K. Hallberg, K.P. Steele, J. Chromatogr. A 744 (1996) 141.
[8] J. Vindevogel, P. Sandra, Introduction to Micellar Electrokinetic Chromatography, Hüthig, Heidelberg, 1992.
[9] I. Miksik, Z. Deyl, J. Chromatogr. A 807 (1998) 111.
[10] R.L. Boso, M.S. Bellini, I. Miksik, Z. Deyl, J. Chromatogr. A 709 (1995) 11.
[11] H. Watarai, I. Takahashi, Anal. Commun. 35 (1998) 289.
[12] J.-D. Lu, X.Y. Fu, X.-Z. Zhu, Chem. J. Chin. Univ. 19 (1998) 1219.
[13] L. Song, Q. Ou, W. Yu, G. Li, J. Chromatogr. A 699 (1995) 371.
[14] M.F. Miola, M.J. Snowden, K.D. Altria, J. Pharm. Biomed. Anal. 18 (1998) 785.
[15] H. Watarai, Chem. Lett. (1991) 391
[16] H. Watarai, J. Chromatogr. A 780 (1997) 93.
[17] K.D. Altria, J. Chromatogr. A 844 (1999) 371.
[18] H. Watarai, K. Ogawa, M. Abe, T. Monta, I. Takahashi, Anal. Sci. 7 (1991) 245.
[19] R. Szücs, E. Van Hove, P. Sandra, J. High Resolut. Chromatogr. 19 (1996) 189.
[20] A. Berthod, M. De Carvalho, Anal. Chem. 64 (1992) 2267.
[21] X. Fu, J. Lu, A. Zhu, J. Chromatogr. A 735 (1996) 353.
[22] G. Li, Z. Chen, M. Liu, Z. Hu, Analyst 123 (1998) 1501.
[23] I. Miksik, J. Gabriel, Z. Deyl, J. Chromatogr. A 772 (1997) 297.
[24] L. Vomastova, I. Miksik, Z. Deyl, J. Chromatogr. B 681 (1996) 107.
[25] J.H. Aiken, C.W. Huie, Chromatographia 35 (1993) 448.
[26] N. Gorski, M. Gradzielski, H. Hoffmann, Ber. Bunsenges. Phys. Chem. 100 (1996) 1109.
[27] R. Szücs, E. Van Hove, P. Sandra, J. High Resolut. Chromatogr. 19 (1996) 189.
[28] K.D. Altria, Chromatographia 49 (1999) 457.
[29] K.D. Altria, B.J. Clark, M.A. Kelly, J. High Resolut. Chromatogr. 22 (1999) 55.
[30] L. Debusschère, C. Demesmay, J.L. Rocca, G. Lachatre, H. Lofti, J. Chromatogr. A 779 (1997) 227.
[31] Y. Mrestani, N. El-Mokdad, H.H. Ruttinger, R. Neubert, Electrophoresis 19 (1998) 2895.
[32] Y. Ishihama, Y. Oda, N. Asakawa, Anal. Chem. 68 (1996) 1028.
[33] Y. Ishihama, Y. Oda, N. Asakawa, Anal. Chem. 68 (1996) 4281.

[34] A. Paulus, A. Klockow-Beck, Analysis of Carbohydrates by Capillary Electrophoresis, Vieweg, Weisbaden, 1999.
[35] E. Dabek-Zlotorzynska, E.P.C. Lai, J. Cap. Electrophoresis 3 (1996) 31.
[36] U. Krismann, W. Kleibohmer, J. Chromatogr. A 774 (1997) 193.
[37] K.D. Altria, N.W. Smith, C.H. Turnbull, Chromatographia 46 (1997) 664.
[38] Y. Ishihama, Y. Oda, K. Uchikawa, N. Asakawa, presented at the 7th International Symposium on High-Performance Capillary Electrophoresis, Würzburg, 29 January–2 February 1995.
[39] G.-H. Zhou, G.-A. Luo, X.-D. Zhang, J. Chromatogr. A 853 (1999) 277.
[40] R. Szücs, J. Videvogel, E. Everaert, L. De Cooman, P. Sandra, D. De Keukeleire, J. Inst. Brew. 100 (1994) 293.
[41] Z. Elrassi, Electrophoresis 18 (1997) 2465.
[42] X. Song, W.L. Buddle, J. Chromatogr. A 829 (1998) 327.
[43] H. Watarai, I. Takahashi, Anal. Commun. 35 (1998) 289.
[44] K.D. Altria, Quantitative Analysis of Pharmaceuticals by Capillary Electrophoresis, Vieweg, Weisbaden, 1998.
[45] H.J. Issaq, Electrophoresis 18 (1997) 2438.
[46] C.M. Boone, J.C.M. Waterval, H. Lingeman, K. Ensing, W.J.M. Underberg, J. Pharm. Biomed. Anal. 20 (1999) 831.
[47] A. Hansa, V.L. Pillay, C.A. Buckley, Water Sci. Technol. 39 (1999) 169.
[48] K. Kitagishi, H. Shintani, J. Chromatogr. B 717 (1998) 327.
[49] K.D. Altria, N.W. Smith, C.H. Turnbull, J. Chromatogr. B 717 (1998) 341.