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REVIEW

MICELLAR, INCLUSION AND METAL-COMPLEX ENANTIOSELECTIVE PSEUDOPHASES IN HIGH-PERFORMANCE ELECTROMIGRATION METHODS

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1. INTRODUCTION

In recent years much effort has been devoted to the development of separation methods especially in the field of analytical chemistry. Since the sixties, the progress in chromatography and related methods has been nearly explosive, connected with the rapid accumulation of experimental material and new information. The present stage can be characterized by the following trends: (1) efforts to improve the separation efficiency and speed of analysis leading to the development and application of high-performance techniques; (2) in order to achieve the widest possible applicability, new hybrid methods involving two or more formerly independent separation methods have been employed; (3) improvement in the separation of structurally related compounds and isomers, especially optical isomers (enantiomers) with the aid of various chiral discriminators.

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These trends are increasingly evident in the electromigration methods (EMMs). The latest and very promising topic is the development of various pseudophases[1] which substantially extend the application of EMMs towards various types of isomers and, on the other hand, to non-electrolytes.

The aim of this review is to cover the most promising modern trends and to characterize the present stage in the utilization of micellar, inclusion and enentioselective metal-complex formation for the creation of pseudophases. This approach may help to characterize better the newly formed types of EMMs.

2. HIGH-PERFORMANCE ELECTROMIGRATION TECHNIQUES

The trend in modern electromigration methods can be characterized by a high resolution and sensitivity achieved in a short time. A major factor in performance of the system is its ability to dissipate the Joule heat generated by high potentials and currents.

The first method in a planar arrangement dates from 1951, when Michl[2] showed how paper electrophoresis (PE) could be speeded up by the use of high potentials (approx. 50 V/cm) and pointed out that the diffusion phenomena in the separation process could thus be considerably decreased. It was possible to separate amino acids, polypeptides, organic acids and metal ions in about 20 min whereas 1–12 h were previously necessary. A rapid electrophoretic method that can yield good separation within a few minutes involving an extremely simple arrangement called "high-performance paper electrophoresis" has been developed by Lederer[3]. Some new and promising elements appeared in his experimental arrangement. The use of a sandwich type separation compartment (paper between two glass plates) minimizes the evaporation of solvent, contributes to a stabilization of the thermal conditions and thus improves substantially the reproducibility of the method.

The introduction of open-tubular capillaries into EMMs represented great progress in the instrumentation. In the past years, high-voltage (HV) or high-performance capillary zone electrophoresis (HPCZE) have become powerful tools for the separation of ionic as well as neutral substances with remarkable efficiency[4–7]. Capillary columns permit high potential fields to be employed to yield high-speed and high-performance separation. A major factor in the performance is the ability of the capillary tubing system to dissipate the Joule heat. In this way, diffusion is decreased and zone-broadening effects are minimized.

The first successful high-performance separation of metal ions in capillary zone electrophoresis mode was carried out by Hjertén[4]. The separation was achieved in about 2–3 min for simple and in 10–20 min for more complicated mixtures, respectively.

A similar acceleration, based on the use of an HV power supply was developed in capillary isotachophoresis (ITP)[8]. The use of an high electric field strength increases the self-sharpening effect which represents an efficient counteraction to diffusion, convection and electroendoosmosis.

The use of aqueous–organic and organic media[9–11] may extend the applicability and efficiency of HPCZE and/or ITP methods to a wide range of compounds which have about the same effective mobilities and pK values and/or are only slightly soluble in water.

As a combination of HPCZE and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)[12], high-performance capillary SDS-PAGE (HPC-SDS-PAGE) has been developed[13]. This method may serve as an example of a non-micellar technique used for the separation and molecular weight determination of peptides and proteins. The SDS is able in general to disrupt all non-covalent bonds. In the case of proteins all multiple non-covalently linked chains are broken and free valences are saturated by SDS. The protein mixture is denaturated and the SDS–polypeptide complexes created are separated via sieving through the polyacrylamide gel matrix[14].

The apparatus for HPCZE may be adapted for isoelectric focusing (IEF). Rapid and reproducible high-performance IEF experiments with human haemoglobin and transferrin were successfully performed in glass capillaries[15]. The experiments confirmed that the IEF resolution under a pH gradient in the capillary separation compartment is very efficient but requires a special postseparation technique for on-line UV detection. This could be achieved either by pumping the zones past the stationary UV detector or by electrophoretic elution.

Electroosmosis, usually performed in a device for HPCZE with extremely thin capillaries[16] or in a commercial capillary ITP device[17], represents a transition between the electrophoretic and liquid chromatographic methods. The electroosmotic effect in thin open tubes is used for pumping the mobile phase. The method could be used in principle for separations of both charged and uncharged species. The resolution of uncharged species depends mostly on their size differences. These differences are usually very small and, consequently, the technique is not very useful for the separation of neutral compounds.

Recently, Terabe *et al.*[18] introduced micellar solubilization into HPCZE and first reported the so-called "micellar electrokinetic capillary chromatography" (MECC). The technique combines many of the operational principles and advantages of micellar liquid chromatography[1] and HPCZE. The surfactant is added to the mobile phase in a concentration above its critical micellar concentration (CMC). The micellar pseudophase formed provides an effective mechanism especially for the separation of neutral hydrophobic compounds. They are resolved in harmony with their partitioning between the mobile phase and the hydrophobic interior of the micelles. An electroosmotic flow of the solvent enables the transport of solutes, including neutral ones, through the separation compartment. The electrokinetic separation seems to be applicable to a variety of non-polar and polar slow migrating solutes. It seems to be limited by the solubility of the solutes in water since the addition of an organic solvent causes a disintegration of the micellar pseudophase.

3. PSEUDOPHASES

There are many possibilities of using the micellar, inclusion and enantioselective complexating phenomena in the electromigration methods. Intensive research in this field was stimulated mainly by the requirement of the resolution of various isomeric compounds, which do not differ in their mobilities and pK values.

In recent years, the interest in chiral separations has grown considerably. The resolution of enantiomers by EMMs, in comparison with chromatographic methods, has just started and is still sporadic. The first successful attempts to separate optical

isomers were made in paper electrophoresis[19], HPCZE[20] and capillary isotachophoresis[21], respectively.

3.1. Micellar pseudophases

The utilization of surface-active agents (surfactants) substantially alters the possibilities and limitations of EMMs and gives rise to the existance of newly modified ones. Most EMMs employ the extraordinary ability of surfactants to form organized aggregates (micelles) and thus may be classified as micellar techniques. Some of the earlier methods do not require the presence of an aggregated surfactant and belong to the non-micellar type. As an excellent example of the widely used non-micellar EMMs, SDS-PAGE[12] must be mentioned, where the presence of the surfactant in micellar form is undesirable. Sometimes it is quite impossible to differentiate micellar and non-micellar techniques because the separation process can be mediated simultaneously by the presence of the micellar and monomeric form of the surfactant.

Many reviews and articles describe the properties of surfactants and their behaviour in aqueous and non-aqueous solutions and propose possible structures for the micelles created[1,22]. Only some fundamental properties of micellar systems, most important for applications in EMMs, will be mentioned here.

The surfactants are amphiphilic molecules containing both hydrophobic and hydrophilic parts. Depending on the charge of the polar "head" group, the surfactant molecule can be classified as anionic, cationic, zwitterionic or non-ionic. The apolar hydrophobic part of the molecule is usually formed by a long alkyl chain, except for some biological surfactants like molecules, namely bile salts, which differ substantially in their structure and micelle formation mechanism[24] and thus must be classified separately.

As suggested above, the surfactants may exist in the form of aggregates when their concentration in solution exceeds the CMC. The CMC value and aggregation number depend on various physicochemical parameters. In EMMs the dependence of the CMC upon the pressure is not significant and may be neglected, but the influence of temperature may substantially alter the separation process. Plots of CMC *vs.* temperature, for the commonly utilized ionogenic surfactants, exhibit a minimum between 20 and 30°C. Dramatic changes in CMC may occur when some electrolytes are added to the surfactant–water system[22]. It can be concluded that an increase in ionic strength often results in an increase in the aggregation number and a decrease in the CMC. Both water-soluble and -insoluble organic solvents may play a significant role in micelle formation[24,25]. Short-chain alcohols added to aqueous micellar systems enhance the micelle formation by decreasing the CMC, if the alcohols are present in low concentration. At higher concentrations they prevent the micelle formation. The organic solvents which are able to form strong hydrogen bonds with water molecules, acetone, dioxane, acetonitrile, tetrahydrofuran, etc., inhibit the formation of micelles. The addition of water immiscible organic solvents like long-chain alcohols or alkanes can either enhance or inhibit micelle formation depending on the concentration of surfactant present in the solution and amount of the organic additive.

The structural description of micellar systems demonstrates the difficult prob-

lem which has not yet been solved in detail. Many simplified models, which contribute to the understanding of micelle properties, have appeared in the literature. The simplest radial model of a normal aqueous micelle[26–28] proposes an approximately spherical geometry with all hydrophobic chains in the central core and head hydrophilic groups situated on the surface (see Fig. 1). This oldest model may serve to explain the solubilization hydrophobic and electrostatic interactions between micelles and solutes in the separation process. Experimental observations, which confirmed a significant contact between water and the hydrocarbon chains of a normal micelle, resulted in the proposal of new and more complicated models. The Menger model of a normal micelle[29–33] supposes that the hydrocarbon chains are extended to the surface and may interact directly with the solvent. The Fromherz model[34] represents an highly organized spherical system with both the hydrocarbon chain portions and the head hydrophilic groups exposed to the solvent. The similar Dill model[35–37], developed recently, is much less structural but again there is a considerable amount of hydrocarbon exposed at the surface of the micelle. These models enable a better explanation of the interspacial surface interactions between micelles and solutes in the separation process.

The shape of micelles depends on the surfactant concentration in the solution. The theoretical models propose a roughly spherical shape for a normal micelle. An increase in the surfactant concentration may result in the formation of other types of aggregates[22,29]. Initially, a transition from spherical to rod-like or cylindrical micelles may be observed. Still higher surfactant concentrations lead to the formation of liquid crystalline aggregates[1,22,38].

The utilization of reversed micellar systems[22,39,40] is connected with the use of apolar organic media. Promising results with reversed micelles in liquid chromatography methods could not be simply transferred to EMMs due to the necessity of using apolar systems with great electric resistance. The only technique which could be compared to the use of reversed-phase liquid chromatography was published by Walbroehl and Jorgenson[41]. The solvophobic interaction between a non-polar solute and a tetraalkylammonium ion utilized is similar to that between a solute and a reversed phase.

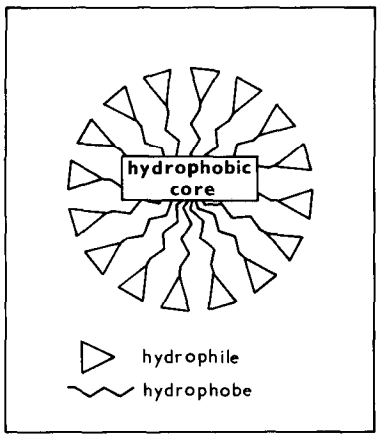

Fig. 1. Radial model of the micelle.

The use of surfactants as pseudophases in EMMs is based on the partitioning of the solute between the micellar phase and the solvent which is a fast dynamic equilibrium process. The distribution constant is defined by the equation

$$K_D = [S]_m/[S]_s$$

where $[S]_m$ = solute concentration in the micellar phase and $[S]_s$ = solute concentration in the solvent phase, respectively.

For diluted solutions the distribution constant may be related to the solute–surfactant binding constant, K_b

$$K_b = [S \cdot M]/[S][M]$$

where [S], [M] and [S · M] are the concentrations of the uncomplexed solute, the micellar pseudophase and the surfactant–solute "complex", respectively, by the use of the equation[42]

$$K_b = (K_D - 1) \cdot \bar{v}$$

where $\bar{v}$ = molar volume of the surfactant in the organized surfactant medium.

As mentioned above, the association rate of the majority of solutes with the surfactant is very rapid and therefore a direct proportionality between the binding constant, K_b, and the stability of the associated solute–surfactant complex can be assumed. The structural differentiation, based on the use of the micellar pseudophase, depends directly on the differences in binding constants of the solutes.

3.2. Inclusion pseudophases

Inclusion complexes are molecular compounds of characteristic structural arrangements, in which one compound (the host molecule) spatically encloses another (the guest molecule) or at least part of it. The inclusion phenomena have found the widest use in separation methods, *e.g.*, in chromatography[22,43–46]. In EMMs, increasing attention has recently been paid to the formation of inclusion compounds of cyclodextrins or its derivatives[21,47–55] and crown ethers[47,56–59]. Both types of compounds are utilized as inclusion pseudophases.

3.2.1. Cyclodextrins

Cyclodextrins (also known as Schardinger dextrins, cycloglucopyranoses, cycloamyloses, cycloglucans)[1] are cyclic oligosaccharide molecules built up of D-(+)-glucopyranose units, bonded via α-(1,4) linkages, with all glucose units in a Cl(D) chair conformation (Fig. 2A). Their structure is unique in that it resembles a truncated cone with both ends open (Fig. 2B). The top of the torus corresponds to the more open side which is rimmed with secondary hydroxyl groups on carbons 2 and 3 of each glucose unit, all rotated to the right. The smaller opening of the cones is rimmed with the more polar primary hydroxyl groups on carbon 6 of the glucose unit. The interior of the cyclodextrin (CD) cavity contains two rings of C–H groups with a ring of glycosidic oxygens in between. Their surface is relatively hydrophilic,

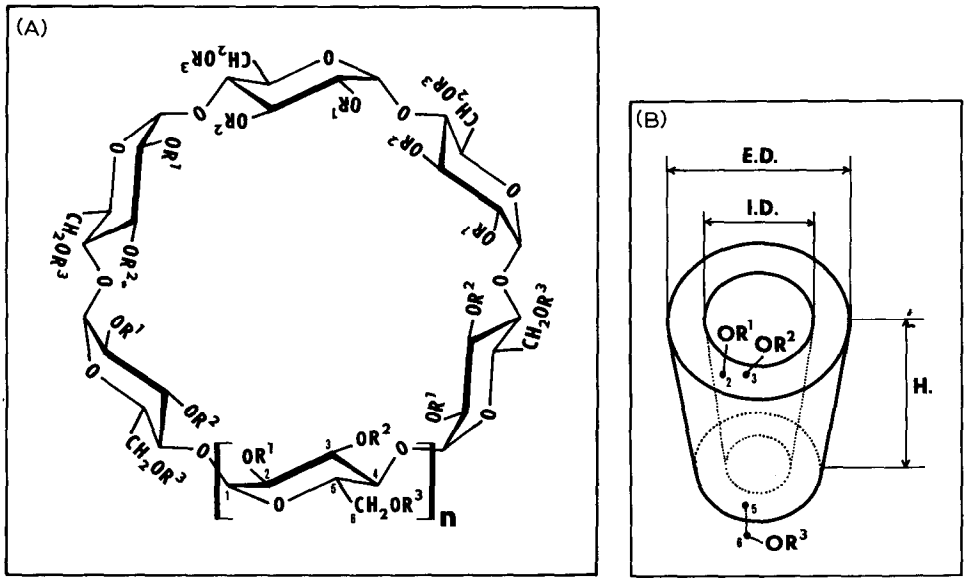

Fig. 2. Chemical structure (A) and schematic model (B) of cyclodextrin and/or its derivatives. For *n*, *H*., I.D., E.D. values and R^{1-3} substitution see Table I.

therefore the CDs are soluble in water. Their cavity, however, is of apolar character. Useful physical data and properties are given in Table I.

The exceptional properties of CDs have been described in many papers, reviews[1,43,44,60] and monographs[22,45,46,61–64]. The remarkable property of CDs, its

TABLE I

CHARACTERISTICS OF CYCLODEXTRINS[1,43,60,61]

	Cyclodextrin		
	α	β	γ
$R^1 = R^2 = R^3$*	H	H	H
Number of glucose units	6	7	8
*n**	1	2	3
Molecular weight	972.86	1135.01	1297.15
Diameter of cavity, I.D.* (nm)	0.47–0.52	0.60–0.64	0.75–0.83
Diameter of outer periphery, E.D.* (nm)	1.46 ± 0.04	1.54 ± 0.04	1.75 ± 0.04
Height of cavity, *H** (nm)		(for all) 0.79–0.80	
Volume of cavity (nm^3)	0.176	0.346	0.510
pK_a range of hydroxyl groups		(for all) 12.1–12.6	
Solubility in water at 25°C (g/100 ml)	14.50	1.85	23.20
Number of water molecules taken up by cavity	6	11	17
Melting point (K)	551	572	540
Specific rotation, $[\alpha]_D^{25}$	150.5 ± 0.5	162.5 ± 0.5	177.4 ± 0.5

* For meaning of abbreviations see Fig. 2.

derivatives and/or polymers is their ability selectively to include a wide variety of guest organic and inorganic molecules or ions into their hydrophobic cavity.

The formation of inclusion compounds of CDs is a spontaneous process with a negative Gibbs energy. It can be described by an equilibrium

$$CD + S \rightleftharpoons CD \cdot S$$

characterized by an equilibrium constant, K_b, defined by the relationship

$$K_b = [CD \cdot S]/[CD][S]$$

where [CD · S], [CD] and [S] are the equilibrium molar concentrations of the inclusion compound, cyclodextrin and guest, respectively. The K_b values indicate the stabilities of the inclusion compounds and the possibilities for the separation of related compounds.

The relative stabilities of the CD inclusion compounds are governed by factors such as hydrogen bonding, hydrophobic interactions (Van der Waals interactions), solvation effects (in liquid media) and the space-filling ability of the molecule. Depending on the size and geometry of the guest molecule, in relation to the dimensions of the CD cavity, substantial differences in binding behaviour can be observed for series of structurally related solutes and optical isomers. This is the basis of the use of CDs in the separation process, including EMMs.

For completeness, some other properties of CDs which may play a significant role in the separation process must be mentioned. Except for inclusion phenomena, the micellar pseudophase formation may occur in water solutions, using derivatized cyclodextrin. The methylated cyclodextrins are known to be extremely soluble in both water and organic solvents, less hygroscopic and highly surface active[65,66]. Their behaviour in aqueous solutions is very similar to that of non-ionic surfactants.

3.2.2. Crown ethers

The crown ethers belong to the group of synthetic macrocyclic polyethers which are able to form stable inclusion complexes with various inorganic and organic cations. Many papers and several monographs, published during the past two decades, have been devoted to aspects of the synthesis and utilization of crown ethers[67–71]. In this review we shall limit the discussion to a brief description of their properties and their use in EMMs as selective pseudophases.

Crown ethers may be classified in general as synthetic macrocyclic compounds containing some electron-donor heteroatoms (O,N,S) in the cyclic structure. This definition includes not only the cyclic oligomers of epoxyalkanes but also derived aromatic and alicyclic crown ethers, thia- and aza-crown ethers which contain sulphur and nitrogen atoms in the ring, respectively.

The structure of a common crown-ether skeleton, with oxygen atoms only, is given in Fig. 3, and the cavity diameters of the simple crown ethers are given in Table II. Many aromatic and alicyclic crown ethers, derived from this fundamental skeleton, with different dimensions and complexation ability, have been synthesized. The crown compounds, *e.g.*, with aromatic or alicyclic units in the fundamental skeleton, are, in comparison to crown ethers of the basic structure, much less soluble in aqueous solutions.

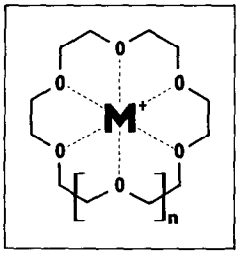

Fig. 3. Chemical structure of a crown–metal (M^+) complex, *e.g.*, $n=1$ for 18-crown-6–M^+, $n=2$ for 21-crown-7–M^+, etc.

Many crown ethers synthesized are able to form complexes not only with metal ions but also with organic nitrocompounds, aryldiazonium salts, amines and nitriles in their ionic forms. The complex formation is based on ion–dipole interactions between the host and guest molecules. The most important factors influencing the stability of crown complexes are summarized in the following paragraphs.

The complex formation depends strongly on the relationships between the cavity diameter and the size of the host ion. The maximum stability is obtained when the cation diameter is comparable with the dimensions of the cavity.

The number, geometrical position and geometrical symmetry of oxygen-donor atoms in the ring are of great importance. The higher is the number of oxygen atoms which lie in the same plane, the more stable is the complex created. A symmetrical arrangement of oxygen atoms is favourable for the stability of the complex.

The partial negative charge of oxygen atoms present in the macrocycle, together with the neighbouring aliphatic carbon atoms, increases the stability of the complex. The presence of aromatic rings in the crown structure decreases the negative charge on the oxygen atoms, thus the stability of the complex is decreased.

Steric hindrance of the polyether ring decreases the stability of the complex formed. On the other hand, the introduction of an optically active group into the crown ether has great significance, especially for the separation of optically active organic compounds. An efficient liquid chromatographic separation of various chiral amino acids has been achieved recently[74–76].

TABLE II

CROWN ETHER CAVITY DIAMETERS[68,72,73]

A, From Corey–Pauling–Koltur atomic models; B, from Fisher–Hirschfelder–Taylor atomic models; C, from X-ray crystallographic data.

Name	*Internal diameter (nm)*		
	A	*B*	*C*
14-Crown-4	0.12	0.15	–
15-Crown-5	0.17	0.22	0.172–0.184
18-Crown-6	0.26	0.32	0.268–0.286
21-Crown-7	0.34	0.43	–
24-Crown-8	0.4		–

Solvation affects substantially the size of the included cation and thus the stability of the complex. It can be concluded that higher solvation of the cation means a less stable complex.

The utilization of crown ethers in EMMs has been restricted to improvement in the separation of alkaline and alkaline-earth metals. The rapid progess in the synthesis of enantioselective crown ethers opens up new possibilities in the separation of enantiomers.

Although the fundamentals of the cyclodextrin and crown-ether inclusion phenomena are rather different, both separation processes can be expressed similarly in terms of complex equilibria between the solvent and pseudophase.

3.3. Enantioselective metal-complex pseudophases

The utilization of enantioselective metal complexes represents one of the oldest approaches to the solution of chiral resolution. The so-called ligand-exchange phases for the separation of enantiomers were introduced in liquid chromatography by Davankov *et al.*[77–79]. An amino acid, such as L-proline, attached to the support material, and Cu^{2+} present in the mobile phase were used for the formation of ternary diastereomeric complexes of different stabilities with the racemic amino acids to be separated and thus an effective chiral resolution was achieved This technique remains one of the most effective means of separating underivatized amino acid racemates.

The diastereomeric ternary complex formation, described above, is theoretically applicable to the resolution of sample molecules with two polar correctly spaced groups which can simultaneously act as ligands for the central metal ion. Therefore, especially α-amino acids with their NH_2 and COOH groups are very suitable for both formation of the enantioselective phase and its utilization as a solute. The structural differentiation of the typical diastereomeric ternary complex, formed by two amino acids as ligands for the central metal ion, is shown in Fig. 4.

The experiments with enantioselective metal complexes were recently transfered to EMMs. The chiral pseudophase may be obtained simply by adding complex-forming components to the support electrolyte without the need of its immobilization. Chiral Cu^{2+}–L-histidine and Cu^{2+}–aspartame pseudophases were successfully used in HPCZE[20,80]. Studies of chiral complexes of Co^{3+} and Cr^{3+} with amino acids, cyclohexanediamine and oxalate as ligands were performed in HPPE[19,81–85]. The detailed description of their behaviour in chiral support electrolytes may help to extend the number of chiral complexes which are of potential use as enantioselective pseudophases.

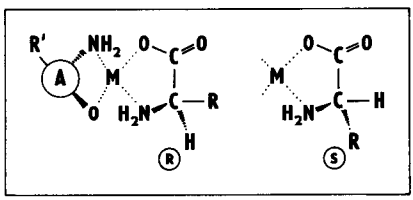

Fig. 4. Schematic model of amino acid chiral resolution by means of amino acid (A)–metal (M) complexes. R = *R*-enantiomer and S = *S*-enantiomer.

4. APPLICATION OF PSEUDOPHASES IN HIGH-PERFORMANCE ELECTROMIGRATION METHODS

The pseudophases described allow a widening of the application range of modern high-performance electromigration methods towards various types of structural isomers (positional, optical, geometrical, etc.) and even uncharged organic compounds. In some cases such modification of the electrophoretic system results in the introduction of quite a new method of separation, *e.g.*, micellar electrokinetic chromatography. The number of theoretical and application contributions, described in the literature, indicates the increasing interest in EMMs.

4.1. HPCZE and its modifications

Most attention has been paid to the application of micellar pseudophases in this area. The modification of HPCZE which utilizes many of the operational principles and advantages of micellar liquid chromatography is frequently called micellar electrokinetic capillary chromatography (MECC). In this method a surfactant is added above its CMC, thereby affording an effective mechanism for the separation of both neutral and charged solutes. The separation of neutral solutes is based simply on their differential partitioning between the electroosmotically pumped aqueous mobile phase and the hydrophobic interior of the micelles, which are charged and moving at a speed slower than the mobile phase due to electrophoretic effects. Charged solutes are not only distributed between the micellar and liquid phases, but are simultaneously separated electrophoretically according to their mobilities. The superposition of the two phenomena may either enhance or deteriorate the separation efficiency. The electrophoretic velocity of charged species must be smaller than the electroosmotic flow in the opposite direction, otherwise there is no possibility to detect and thus quantify the moving zone.

The use of other types of pseudophases in electrokinetic chromatography is based on the same principles mentioned above and may further extend its application area. The use of 2-O-carboxymethyl-β-CD as a pseudophase permits the distribution process between the mobile phase and the CD cavity. This modification seems to be advantageous especially for the separation of neutral aromatic isomers.

The creation of a metal-complexing pseudophase in the support electrolyte, *e.g.*, of the Davankov type, enables the diastereomeric chiral discriminating complex formation between the pseudophase and the solutes. The enantiomeric resolution of positively and negatively charged solutes can be achieved in principle.

The applications of pseudophases in HPCZE, described in literature, are summarized in Table III.

4.2. Capillary isotachophoresis

The use of pseudophases in capillary ITP is in the development stage. Most attention has been paid to the utilization of macrocyclic ionophores such as crown ethers and cyclodextrins. The mechanism of the separation process in ITP differs from that in CZE. The electroendoosmotic flow, which plays a significant role in CZE, is minimized here and we may presume that the uncharged inclusion pseudo-

TABLE III

APPLICATION OF PSEUDOPHASES IN HPCZE

Abbreviations used: β-Ala = 3-alanine, C = capillary, coumarin 540 A = tetrahydro(trifluoromethyl)benzopyrazolizin-11-one, β-CMCD = 2-O-carboxymethyl-β-cyclodextrin, CTAC = cetyltrimethylammonium chloride, D = detection, DE = device, DTAB = dodecyltrimethylammonium bromide, E = electrolyte, EACA = 6-aminocaproic acid, GD = Girard derivative, $(h_i)_{rel}$ = relative step height, HEC = hydroxyethylcellulose, His = L-histidine, HPMC = hydroxypropylmethylcellulose, LE = leading electrolyte, MES = morpholinoethanesulphonic acid, NBD = 7-nitrobenzofurazan, *n*-CA = *n*-caproic acid, PTH = phenylthiohydantoin, PU = potential units, PVA = poly(vinyl alcohol), STS = sodium tetradecyl sulphate, TBAB = tetrabutylammonium bromide, TBAC = tetrabutylammonium chloride, TE = terminating electrolyte, TEA^+ = tetraethylammonium, Tris = tris(hydroxymethyl)aminomethane, Val = L-valine.

Pseudophases	*Conditions*	*Compounds separated*	*Notes*	*Refs.*
SDS	E: borate–phosphate buffer (pH 7.0) C: silica tubing (650 or 900 mm × 0.05 mm I.D.) D: UV (270 nm)	Acetylacetone, phenol, *o*-, *m*-, *p*-cresols, chlorophenols, xylenols, *p*-ethylphenol	Max. theoretical plate number 400 000 for *p*-ethylphenol and xylenols (height equivalent to a theoretical plate of 1.9–3.6 μm)	18
SDS, STS	E: borate–phosphate buffer (pH 7.0) C: silica tubing (500 mm × 0.05 mm I.D.) D: UV (210 nm)	Methanol, resorcinol, phenol, *p*-nitroaniline, nitrobenzene, toluene, 2-naphthol, Sudan III	Fundamental characteristic of MECC; the mathematical description of capacity factor, resolution, effective plates and peak capacity; Range of retention times of electrically neutral solutes are limited between elution time of water, t_0, and that of the micelle, t_{mc}	86
SDS, DTAB	E: borate–phosphate buffer (pH 7.0) C: fused-silica tubing (650 mm × 0.05 mm I.D.) D: UV (260 nm)	22 PTH-amino acids	Comparative measurements with anionic SDS and cationic DTAB were performed	87
SDS	E: borate–phosphate buffer (pH 6.0–9.0) C: fused-silica tubing (650 mm × 0.05 mm I.D.) D: UV (220 nm)	Chlorinated phenols (mono-, di-, tri-, tetra-, penta-), phenol, Yellow OB	The capacity factor of chlorinated phenols, which were partially ionized under given conditions, decreases with increasing pH	88
SDS	E: borate–phosphate buffer (pH 5.0–9.0) C: fused-silica tubing (650 mm × 0.05 mm I.D.) D: UV (210 nm)	Methanol, resorcinol, phenol, *p*-nitroaniline, nitrobenzene, toluene, 2-naphthol, Sudan III	Coating of polymers polyethylene glycol 20M (DB-WAX), methylsilicone (DB-1) on the inner wall of fused-silica tubing; effect of pH and concn. of SDS on electrokinetic migration were investigated	89

TABLE III *(continued)*

Pseudophases	*Conditions*	*Compounds separated*	*Notes*	*Refs.*
SDS	E: phosphate–borate buffer C: 25–75 μm I.D. D: laser-excited fluorescence	B_6 vitamin and its five metabolites	Determined in human urine; efficiency as high as 60 000 theoretical plates/m; limits of detection are less than 1 pg injected	90
SDS	E: borate–phosphate buffer C: fused-silica (1 m × 75 μm I.D.) D: UV (280 nm)	Theobromine, hypoxanthine, theophylline, caffeine, uric acid, xanthine	Effect of injection procedures on efficiency in MECC; 240 000 theoretical plates/m are reported for caffeine	91
SDS, CTAC	E: soln. Na_2HPO_4 C: fused-silica (650 mm × 0.05 mm I.D.) D: fluorescence (488 nm/540 nm)	NBD-ethylamine, -*n*-propylamine, -*n*-butylamine, -cyclohexylamine, -*n*-hexylamine, coumarin 540A	Inside wall of the column is silanated by trimethylchlorosilane. The effect of 2-propanole on diminution of solute–wall interaction and separation efficiency was studied	92
SDS	E: borate–phosphate buffer (pH 7.0) C: quartz (700 mm × 0.05 mm I.D.) D: UV (206 nm)	Phenol, PTH-alanine, 4-nitroaniline, di-, triglycine, (*S*)-*cis*-verbenol, GD of nonanol and undecanol, (*E*)-9-dodecen-1-ol, (8*E*, 10*E*)-8,10-dodecadien-1-ol, 1- and 2-naphthol	Inside wall of the column coated by Sylgard 184 (dimethylpolysiloxane RTV). The experimental device was constructed utilizing the components of capillary ITP	93
SDS	E: phosphate buffer (pH 7.0–11.0) C: fused silica (800 mm × 0.1 mm I.D.) D: UV (214 nm)	Acetylsalicylic acid, caffeine, *p*-acetamidophenol, salicylamide, *o*-ethoxybenzamide	Ethyl *p*-aminobenzoate was used as the internal standard for on-column detection. Column efficiency: 70 000–130 000 theoretical plates.	94
SDS	E: borate–phosphate buffer (pH 7.0) C: fused silica (650 mm × 0.05 mm I.D.) D: UV	Chlorinated phenols (mono-, di-, tri-, tetra-, penta-), methanol, Yellow OB, resorcinol, *p*-nitroaniline, nitrobenzene, 2-naphthol	Phenol was used as internal standard. Reproducibility of retention times, peak area and peak heights for repeated injection of constant amounts of samples is presented.	95
SDS ($+Zn^{2+}$, Mg^{2+}, Cu^{2+})	E: borate–phosphate buffer (pH 7–9) C: fused silica (500–850 mm × 0.05 mm I.D.) D: UV (260 nm)	Eight bases, nucleotides, 18 oligonucleotides	Combination of low concentration of divalent metals and SDS micelles is demonstrated.	96
SDS	E: Na_2HPO_4 solutions C: fused silica (750 mm × 0.025, 0.075 and 0.10 mm I.D.) D: fluorescence (480/540 nm)	NBD-cyclohexylamine, ethylamine	Effects of the applied voltage, column dimensions, concentration of buffer and surfactant on the separation efficiency were studied.	97

(Continued on p. 584)

TABLE III *(continued)*

Pseudophases	*Conditions*	*Compounds separated*	*Notes*	*Refs*
SDS	E: borate–phosphate buffer (pH 8.4–8.9) C: fused silica (685 mm × 0.06 mm I.D.) D: UV (256 nm)	Fourteen normal and modified deoxyribonucleosides, deoxyribomononucleotides, ribonucleoside and pyrimidine	The number of theoretical plates: 370 000–540 000 (highly dependent on the solute concentration). The dependence of the applied voltage and electroinjection time on the separation efficiency was studied.	98
SDS	E: phosphate buffer (pH 6.95–7.0) MES buffer (pH 6.05) C: fused silica (879 mm × 0.026 mm I.D. and 651 mm × 0.052 mm I.D.) D: amperometric	Dopamine, norepinephrine, epinephrine, catechol, 4-methyl- and 4-ethylcatechol	New type of detection for MECC was developed. The detection limit was less than 20 fmol. The number of theoretical plates was more than 400 000.	105
β-CMCD β-CD	E: phosphate buffer (pH 7.0) C: silica tubing (650 mm × 0.05 mm I.D.) D: UV (210 nm)	Acetophenone, anisole, methyl-, ethyl-, propyl-, butyl-benzoate, cresols, nitroanilines, chloroanilines, dinitrobenzenes, nitrophenols, xylidines, xylenols	Host–guest interactions between β-CMCD and the solute operates as distribution process. This concept extends the utilization of specific interactions in electrokinetic chromatography. The number of theoretical plates are 120 000–130 000.	55
Cu^{2+}–L-histidine complex	E: L-histidine, $CuSO_4$, ammonium acetate (pH 7–8) C: fused silica (750 mm × 0.075 mm I.D.) D: laser fluorescence (325 nm)	Ten enantiomers of DL-dansyl amino acids	Chiral recognition is explained by mixed chelate complexation to form two diastereomeric, ternary complexes. Replacement of L- by D-His in the support E reverses the migration order of the D- and L- amino acids.	20
Cu^{2+}–aspartame complex STS	E: aspartame, $CuSO_4$, ammonium acetate (pH 7.5) C: fused silica (1 m × 75 μm I.D.) D: laser-induced fluorescence (325/550 nm)	Eighteen racemic dansylated DL-amino acids	Aspartame = dipeptide L-aspartyl-L-phenylalanine methyl ester. The separation is based on the diastereomeric interaction between DL-amino acids and chiral Cu^{2+}–aspartame complex present in the support electrolyte. Effect of electrolyte composition, pH and temperature are described as well as linearity and sensitivity of response. Micellar (STS) electrolyte solution has been employed in order to increase the differentiation of neutral amino acids.	80

TABLE III *(continued)*

Pseudophases	*Conditions*	*Compounds separated*	*Notes*	*Refs.*
			Review, univ. microfilms, etc.	99–104
Mixed micelles didecyl-L-Ala + SDS	E: Sodium acetate, SDS,*N*,*N*-didecyl-L-Ala, Cu^{2+}, 10% glycerol C: 800 mm × 0.075 mm I.D. D: UV (260 nm)	Dansylated D,L-threonine, -methionine and -leucine	Overviews several aspects of HPCZE and presents chiral resolution of dansylated amino acids using a chiral metal chelate micelle.	104

TABLE IV

APPLICATION OF PSEUDOPHASES IN ITP

For abbreviations used, see Table III. HEPES = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

Pseudophases	*Conditions*	*Compounds separated*	*Notes*	*Refs.*
α-CD 18-crown-6 (12-crown-4 and 15-crown-5)	DE: Shimadzu IP-2A C: 40 mm × 1 mm I.D. + 150 mm × 0.5 mm I.D. LE: 5 m*M* *p*-toluenesulphonic acid, 0.01% Triton X-100 TE: 5 m*M* TBAB	Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+, TEA^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, I^-, Cl^-, Br^-, ClO_4^-, $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$	TEA^+ was used as an internal standard. Li^+, Mg^{2+}, Ca^{2+} and TEA^+ do not interact with 18-crown-6. 12-crown-4 and 15-crown-5 were practically without any effect on the electrophoresis. 2% α-CD allowed the otherwise impossible separation of I^- in the presence of Cl^- and Br^-. 1% α-CD allowed the separation of ClO_4^- in the presence of ferri- and ferro-cyanide.	47
β-CD	DE: Shimadzu IP-2A C: 40 mm × 1 mm I.D. + 150 mm × 0.5 mm I.D. LE: 10 m*M* HCl, β-Ala (pH 3.6) TE: 10 m*M* *n*-CA	*o*-, *m*-, *p*-Iodobenzenesulphonate, toluenesulphonate, naphthalene-mono- and di-sulphonate	The dependence of PU values on β-CD concentration in the LE was studied.	49
β-CD	DE: Shimadzu IP-2A C: 40 mm × 1 mm I.D. + 150 mm × 0.5 mm I.D. Acidic electrolyte systems (pH 3.5–4.0)	*o*-, *m*- and *p*-Substituted benzenesulphonic acids (BSA):NH_2-, OH-, MeO-, EtO-, Me-, Cl-, I-; naphthalenesulphonic acids (NSA)	Me-, Cl-, I-BSA and NSA can be separated effectively in the LE with β-CD.	50

(Continued on p. 586)

TABLE IV *(continued)*

Pseudophases	*Conditions*	*Compounds separated*	*Notes*	*Refs.*
α-CD	DE: Shimadzu IP-2A C: 150 mm × 0.5 m I.D. LE: 10 mM HCl or acetic acid + potassium acetate (1:1), 0.01% PVA TE: 5 m*M* (*p*-methylbenzyl)trimethylammonium bromide or 1-naphthylamine hydrochloride	Li^+, Na^+, K^+, TEA^+ (*o*-, *m*- and *p*-methylbenzyl)-trimethylammonium ions, (*o*- and *p*-nitrobenzyl)-trimethylammonium ions, (*m*-, *p*- and *o*-, *p*-dimethylbenzyl)trimethylammonium ions, quaternary pyridinium ions	Li^+, Na^+, K^+, TEA^+ = calibration ions. The evaluation of the binding equilibrium constants between α-CD and investigated ions.	48
α-, β- and γ-CD	DE: LKB Tachophor 2127 C: 400 mm × 0.5 mm I.D. or 520 mm × 0.5 mm I.D. LE: 5 m*M* HCl + EACA (pH 4.3–4.8) or β-Ala (pH 3.5–4.0), 0.2% HPMC TE: 5 m*M* MES or *n*-CA	Related penicillins (sulbactam, its synthetic intermediates and alkaline degradation product)	Comparative measurements with D-glucose and starch were carried out to confirm the role of the cyclic structure of CD for separation improvement. The most important factors are: CD ring diameter, pH and CD concentration in LE.	51
α-, β- and γ-CD	DE: LKB Tachophor 2127 C: 400 mm × 0.5 mm I.D. LE: 5 mM HCl, EACA (pH 4.7), 0.2% HPMC TE: 5 mM MES	*o*-, *m*-, *p*-Halogenobenzoic acids: F-, Cl-, Br- and I-	The dependence of $(h_i)_{rel}$ values on the concentration of CDs, CD cavity diameter and size of substituents was demonstrated.	52
β-CD	DE: LKB Tachophor 2127 C: 400 mm × 0.5 mm I.D. LE: I, 5 m*M* sodium acetate, acetic acid (pH 5.5), 0.2% HPMC; II, 5 m*M* HCl, His (pH 5.0), 0.2% HPMC TE: I, 10 m*M* EACA; II, 5 m*M* MES	Naftidrofuryl and its synthesis intermediates positional isomers	The study of axial and equatorial inclusion complex formation in both cationic and anionic ITP mode. The possibility of quantitative evaluation and purity control of final sunstances is demonstrated,.	53
β-CD, diMe-β-CD, triMe-β-CD	DE: LKB Tachophor 2127 C: 370 mm × 0.5 mm I.D. or 250 mm × 0.5 mm I.D. LE: I, 5 m*M* sodium acetate, acetic acid (pH 5.48), 0.2% HEC; II, 5 m*M* $Ba(OH)_2$, Val (pH 9.69), 0.2% HEC TE: I, 10 m*M* β-Ala; II, 20 m*M* Tris	Ephedrine alkaloid enantiomers	Chiral ITP resolution of pseudoephedrines is demonstrated. The dependence of the chiral resolution quality on the type and concentration of CD was studied.	21

TABLE IV *(continued)*

Pseudophases	*Conditions*	*Compounds separated*	*Notes*	*Refs.*
β-CD, diMe-β-CD	DE: LKB Tachophor 2127 C: 370 mm × 0.5 mm I.D. LE: 5 m*M* sodium acetate, acetic acid (pH 5.5), 0.2% HEC TE: 10 m*M* β-Ala	Ketotifen and its polar intermediate	The discovery of ketotifen enantiomers; the dependence of maximum racemate loads on the type and concentration of CD in the LE was studied. The possibility of ketotifen's quantitative evaluation is confirmed by statistically processed calibration data.	54
α, β- and γ-CD, diMe-β-CD, triMe-β-CD	DE: LKB Tachophor 2127 C: 230 mm × 0.5 mm I.D. or 370 mm × 0.5 mm I.D. LE: 5 mM HCl + His (pH 6.4), 0.4% HEC TE: 10 mM HEPES + Tris (pH 8.3)	17 bile acids	The study of solubilization and structural differentiation effect onCDs.	106
18-crown-6	DE: LKB Tachophor 2127 C: 200 mm × 0.8 mm I.D. LE: 10 m*M* HCl TE: 10 m*M* Tris or TBAC	Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+ and lysine	The simultaneous determination of metal ions in 18-crown-6-modified LE. Simplified mathematical model for the evaluation of stability constants for 1:1 crown–metal complexes is presented. The monitoring of lysine neutralization with KOH was performed.	56,57
18-crown-6	DE: non-commercial LE: $CsNO_3$, HNO_3 (pH 4.4–4.5) 0.05% PVA TE: 5 m*M* acetic acid	Na^+, K^+, Cs^+, Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Al^{3+}	The method for metal ion analysis in glass is demonstrated.	58

phase does not migrate and acts simply as a pseudostationary phase. This experimental arrangement is advantageous especially for the separation of rapidly migrating organic ions. The deliberate use of micellar and enantioselective metal-complex pseudophases for the improvement of separation has not been reported.

Crown ethers were utilized for the selective retardation of inorganic ions, especially for the differentiation of alkaline and alkaline-earth metals.

Cyclodextrins seem to be useful especially in the area of structurally related organic compounds and also inorganic anions. In ITP the cyclodextrins are free in the solution and therefore inclusion complex formation should be the main resolution mechanism. The increasing number of cyclodextrin derivatives, which are commercially available, greatly extend the application area of ITP to organic isomeric compounds, including enantiomers, in their cationic as well as anionic forms. Several other parameters affect the resolution, such as the concentration of the pseudophase, pH value and type of counter ion. The possible applications of ionophore pseudophases are summarized in Table IV.

5. CONCLUSIONS

The use of various pseudophases in EMMs represents one of the most promising approaches to increasing the resolution power of the latter and the extension of their applications especially to organic molecules. The application of new types of pseudophases is determined by the rapid progress in the theory of inclusion and micellar phenomena and steadily increasing number of suitable carrier molecules and tenzides.

The utilization of pseudophases enables the introduction of another important factor which may contribute to improvement in separations. The experience with micellar pseudophases has enabled to the applicability of electromigration methods to be extended to uncharged organic molecules. The use of inclusion pseudophases significantly improves the differentiation of structurally related organic solutes. There is no sharp boundary between micellar and inclusion pseudophases. It was experimentally confirmed that derivatized cyclodextrins form organized systems in aqueous solutions and thus may act also as micellar pseudophases.

The use of optically active pseudophases is the only way of separating enantiomers. Promising results with cyclodextrins in ITP has demonstrated their usefulness as chiral discriminators for the separation of aromatic solutes. Copper–amino acid complexes were utilized for the separation of various α-amino acids in capillary electrophoresis. New possibilities exist for the use of newly synthesized chiral crown ethers and chiral micellar systems in high-performance electromigration methods.

SUMMARY

An overview of important trends in electromigration methods, with emphasis on development of highly efficient techniques and application of various complex-forming discriminators, is presented. The fundamental characteristics of micellar, inclusion and metal-complex pseudophases and their utilisation in high-performance electromigration methods are discussed in detail. Their advantages are demonstrated on numerous practical examples.

REFERENCES

1 D. W. Armstrong, *Sep. Purif. Methods*, 14 (1985) 213.
2 H. Michl, *Monatsh. Chem.*, 82 (1951) 489.
3 M. Lederer, *J. Chromatogr.*, 171 (1979) 403.
4 S. Hjertén, *Chromatogr. Rev.*, 9 (1967) 122.
5 F. E. P. Mikkers, F. M. Everaerts and T. P.E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.
6 J. W. Jorgenson and K. DeArman Lukacs, *Anal. Chem.*, 53 (1981) 1298.
7 S. Hjertén, *J. Chromatogr.*, 270 (1983) 1.
8 F. M. Everaerts, J. L. Beckers and T. P. E. M. Verheggen, *Isotachophoresis*, Elsevier, Amsterdam, 1976.
9 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 51 (1970) 339.
10 Y. Walbroehl and J. W. Jorgenson, *J. Chromatogr.*, 315 (1984) 135.
11 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 487.
12 L. R. Sherman and J. A. Goodrich, *Chem. Soc. Rev.*, 14 (1985) 225.
13 A. S. Cohen and B. L. Karger, *J. Chromatogr.*, 397 (1987) 409.
14 A. Chrambach, in V. Neuhoff and A. Maelicke (Editors), *Advanced Methods in the Biological Science*, VCH Verlagsgesellschaft, Weinheim, 1985, Ch. 15, p. 177.

15 S. Hjertén and M.-D. Zhu, *J. Chromatogr.*, 346 (1985) 265.
16 T. Tsuda, K. Nomura and G. Nakagawa, *J. Chromatogr.*, 248 (1982) 241.
17 T. Tsuda, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 622.
18 S. Terabe, K. Otsuka, K. I. Chikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
19 H. Yoneda and T. Miura, *Bull. Chem. Soc. Jpn.*, 43 (1970) 574.
20 E. Gassmann, J. E. Kuo and R. N. Zare, *Science, (Washington, D.C.)*, 230 (1985) 813.
21 J. Snopek, I. Jelínek and E. Smolková-Keulemansová, *J. Chromatogr.*, 438 (1988) 211.
22 W. L. Hinze and D. W. Armstrong (Editors), *Ordered Media in Chemical Separation, ACS Symp. Ser.*, 342), American Chemical Society, Washington, DC, 1987.
23 D. W. Armstrong, *Am. Lab. (Fairfield, Conn.)*, 13 (1981) 14.
24 C. A. Burton, L. H. Gan, F. H. Hamed and J. R. Moffatt, *J. Phys. Chem.*, 87 (1983) 336.
25 M. Manabe, Y. Tanizaki and H. Watanabe, *Niihama Kogyo, Koto Semmon Gakko Kiyo, Rikogaku Hen*, 19 (1983) 50; *C.A.*, 98 (1983) 204875x.
26 G. S. Hartley, *Trans. Faraday Soc.*, 31 (1935) 31.
27 G. S. Hartley, *Q. Rev. Chem. Soc.*, 2 (1948) 152.
28 P. Mukerjee and J. R. Cardinal, *J. Phys. Chem.*, 82 (1978) 1620.
29 F. M. Menger, *Acc. Chem. Res.*, 12 (1979) 111.
30 F. M. Menger, J. M. Jerkunica and J. C. Johnston, *J. Am. Chem. Soc.*, 100 (1978) 4676.
31 F. M. Menger aand B. J. Boyer, *J. Am. Chem. Soc.*, 102 (1980) 5936.
32 F. M. Menger and J. M. Bonicamp, *J. Am. Chem. Soc.*, 103 (1981) 2140.
33 F. M. Menger, K. S. Venkatasubban and A R. Das, *J. Org. Chem.*, 46 (1981) 415.
34 P. Fromherz, *Chem. Phys. Lett.*, 77 (1980) 460.
35 K. A. Dill and P. J. Flory, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 3115.
36 K. A. Dill and P. J. Flory, *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 676.
37 K. A. Dill, D. E. Koppel, R. S. Cantor, J. D. Dill, D. Bendedouch and S.-H. Chen, *Nature (London)*, 309 (1984) 42.
38 H. Wennerstrom and B. Lindman, *Phys. Rep.*, 52 (1979) 1.
39 H. F. Eicke, *Pure Appl. Chem.*, 53 (1981) 1417.
40 N. J. Muller, *J. Colloid Interface Sci.*, 63 (1978) 383.
41 Y. Walbroehl and J. W. Jorgenson, *Anal. Chem.*, 58 (1986) 479.
42 I. V. Berezin, K. Martinek and A. K. Yatsiminsky, *Russ. Chem. Rev.*, 42 (1973) 778.
43 E. Smolková-Keulemansová, *J. Chromatogr.*, 251 (1982) 17.
44 E. Smolková-Keulemansová and S. Krýsl, *J. Chromatogr.*, 184 (1980) 347.
45 D. Sybilska and E. Smolková-Keulemansová, in J. L. Atwood, J E. D. Davies and D. D. Mac Nicol (Editors), *Inclusion Compounds*, Vol. 3, Academic Press, London, Orlando, San Diego, San Francisco, New York, Toronto, Montreal, Sydney, Tokyo, Sao Paulo, 1984, p. 173.
46 E. Smolková-Keulemansová, in D. Duchêne (Editor), *Cyclodextrins and Their Industrial Uses*, de Santé, Paris, 1987.
47 M. Tazaki, M. Takagi and K. Ueno, *Chem. Lett.*, 5 (1982) 639.
48 M. Tazaki, T. Hayashita, Y. Fujino and M. Takagi, *Bull. Chem. Soc. Jpn.*, 59 (1986) 3459.
49 N. Kuramoto, *Chem. Express*, 1 (1986) 343.
50 N. Kuramoto and K. Asao, *Osaka-Furitsu Kogyo Gijutsu Kenkyusho Hokoku* 88, (1986) 18, *C.A.*, 106, (1987) 188186z.
51 I. Jelínek, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 405 (1987) 379.
52 J. Snopek, I. Jelínek and E. Smolková-Keulemansová, *J. Chromatogr.*, 411 (1987) 153.
53 I. Jelínek, J. Dohnal, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 435 (1988) 496.
54 I. Jelínek, J. Snopek and E. Smolkovà-Keulemansová, *J. Chromatogr.*, 439 (1988) 386.
55 S. Terabe, H. Ozaki, K. Otsuka and T. Ando, *J. Chromatogr.*, 332 (1985) 211.
56 F. S. Stover, *J. Chromatogr.*, 298 (1984) 203.
57 F. S. Stover, *J. Chromatogr.*, 368 (1986) 476.
58 F. M. Everaerts and J. W. Venema, *Isotachophorese als Mogelijke Analyse Methode voor "Glasgemengen"*, Report, Technical University, Eindhoven, 1987.
59 F. M. Everaerts, personal communication.
60 S. Krýsl and E. Smolková-Keulemansová, *Chem. Listy*, 79 (1985) 919.
61 J. Szejtli, *Cyclodextrins and their Inclusion Complexes*, Akademiai Kiados, Budapest, 1982.
62 F. Cramer, *Einschlussverbindungen*, Springer, Berlin, Göttingen, 1954.
63 L. Mandelcorn, *Non-Stoichiometric Compounds*, Academic Press, New York, 1964.

64 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, Berlin, Heidelberg, New York, 1978.
65 K. Uekama and T. Irie, in D. Duchene (Editor), *Cyclodextrins and Their Industrial Uses*, de Santé, Paris, 1987.
66 K. Uekama, personal communication.
67 C. J. Pedersen, *J. Am. Chem. Soc.*, 89 (1967) 7017.
68 C. J. Pedersen, *J. Am. Chem. Soc.*, 92 (1970) 386.
69 C. J. Pedersen, *J. Am. Chem. Soc.*, 92 (1970) 391.
70 C. J. Pedersen and J. K. Frensdorf, *Angew. Chem.*, 84 (1972) 16.
71 M. Hiraoka, *Crown Compounds —their Characteristics and Applications*, Elsevier, Amsterdam, Oxford, New York (Russian translation, Moscow, Mir, 1986).
72 K. D. Pedersen and Ch. K. Frensdorf, *Usp. Khim.*, 42 (1973) 493.
73 N. K. Dalley, in R. M. Izatt and J. J. Christensen (Editors), *Synthetic Multidentate Macrocyclic Compounds*, Academic Press, New York, London, 1978, Ch. 4, p. 207.
74 L. R. Sousa, G. D. Y. Sogah, D. H. Hoffman and D. J. Cram, *J. Am. Chem. Soc.*, 100 (1978) 4569.
75 G. D. Y. Sogah and D. J. Cram, *J. Am. Chem. Soc.*, 101 (1979) 3035.
76 T. Shinbo, T. Yamaguchi, K. Nishimura and M. Sugiura, *J. Chromatogr.*, 405 (1987) 145.
77 V. A. Davankov and S. V. Rogozhin, *J. Chromatogr.*, 60 (1971) 280.
78 V. A. Davankov, S. V. Rogozhin, A. V. Semechkin and T. P. Sachkova, *J. Chromatogr.*, 82 (1973) 359.
79 V. A. Davankov, A. A. Kurganov and A. S. Bochkov, *Adv. Chromatogr.*, 22 (1983) 71.
80 P. Gozel, E. Gassmann, H. Michelsen and R. N. Zare, *Anal. Chem.*, 59 (1987) 44.
81 L. Ossicini and C. Celli, *J. Chromatogr.*, 115 (1975) 655.
82 S. Fanali and L. Ossicini, *J. Chromatogr.*, 212 (1981) 374.
83 S. Fanali, V. Cardaci and L. Ossicini, *J. Chromatogr.*, 265 (1983) 131.
84 S. Fanali, L. Ossicini and T. Prosperi, *J. Chromatogr.*, 318 (1985) 440.
85 S. Fanali, P. Masia and L. Ossicini, *J. Chromatogr.*, 403 (1987) 388.
86 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
87 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 332 (1985) 219.
88 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 348 (1985) 39.
89 S. Terabe, H. Utsumi, K. Otsuka, T. Ando, T. Inomata, S. Kuze and Y. Hanaoka, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 666.
90 D. E. Burton, M. J. Sepaniak and M. P. Maskarinec, *J. Chromatogr. Sci.*, 24 (1986) 347.
91 D. E. Burton, M. J. Sepaniak and M. P. Maskarinec, *Chromatographia*, 21 (1986) 583.
92 A. T. Balchunas and M. J. Sepania, *Anal. Chem.*, 59 (1987) 1466.
93 Z. Prusík, V. Kašička, S. Staněk, G. Kuncová, M. Hayer and J. Vrkoč, *J. Chromatogr.*, 390 (1987) 87.
94 S. Fujivara and S. Honda, *Anal. Chem.*, 59 (1987) 2773.
95 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 396 (1987) 350.
96 A. S. Cohen, S. Terabe, A. J. Smith and B. L. Karger, *Anal. Chem.*, 59 (1987) 1021.
97 M. J. Sepaniak and R. O. Cole, *Anal. Chem.*, 59 (1987) 472.
98 K. H. Row, W. H. Griest and M. P. Maskarinec, *J. Chromatogr.*, 409 (1987) 193.
99 K. Hattori, *Bio Ind.*, 4 (1987) 327; *C.A.*, 107 (1987) 167984p.
100 Y. Walbroehl, *Theoretical and Practical Aspects of Electrokinetic Separation Techniques in Open Tubular Capillaries*, Univ. Microfilms Int., Univ. North Carolina, Chapel Hill, NC, U.S.A.; Order No. DA 8628270, 1986; *C.A.*, 106 (1987) 108504p.
101 D. E. Burton, *Development and Application of Micellar Electrokinetic Capillary Chromatography*, Univ. Microfilms Int., Univ. Tennessee, Knoxville, TN, U.S.A.; Order No. DA 8713452, 1987; *C.A.*, 107 (1987) 171953v.
102 M. J. Sepaniak, D. E. Burton and M. P. Maskarinec, *ACS Symp. Ser.*, 342 (1987) 142.
103 J. H. Knox and I. H. Grant, *Chromatographia*, 24 (1987) 135.
104 A. S. Cohen, A. Paulus and B. L. Karger, *Chromatographia*, 24 (1987) 15.
105 R. A. Wallingford and A. G. Ewing, *Anal. Chem.*, 60 (1988) 258.
106 J. Snopek, E. Smolková-Keulemansová, I. Jelínek, J. Dohnal, J. Klinot and E. Klinotová, *J. Chromatogr.*, in press.