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Review

Micelle polymers, polymer surfactants and dendrimers as pseudo-stationary phases in micellar electrokinetic chromatography

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Abstract

Several authors have recently reported the use of micelle polymers, polymer surfactants and dendrimers as pseudo-stationary phases in electrokinetic chromatography. These reports have demonstrated the effectiveness of these phases for a variety of applications, including the separation and analysis of hydrophobic compounds and chiral compounds and the application of mass spectrometric detection. This review covers developments in this area since the first introduction of polymeric pseudo-stationary phases in 1992. The use of polymeric micelles in electrokinetic chromatography is compared briefly with capillary electrochromatography. Some thoughts on future directions in this area are presented. © 1997 Elsevier Science B.V.

Keywords: Reviews; Polymer pseudo-stationary phases; Stationary phases, pseudo; Enantiomer separation

Contents

1. Introduction

Introduced in 1984 by Terabe et al. [1], micellar electrokinetic chromatography (MEKC) is a modification of capillary electrophoresis (CE) which separates charged or neutral compounds based on their relative affinity for the lipophilic interior and/or the ionic exterior of a micellar pseudo-stationary phase. Due to electrophoretic effects, negatively charged micelles formed from anionic surfactants

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such as sodium dodecyl sulphate (SDS) migrate at a rate slower than that of the electroosmotic flow. The rate of migration of an analyte therefore depends on its partition coefficient between the micelles and the electroosmotically pumped aqueous phase. This has proven to be a powerful tool for the separation and analysis of a variety of analytes (e.g., [2–5]). Micelles and MEKC are reviewed elsewhere in this volume.

The migration of an analyte can be described mathematically in MEKC as follows. The equation for the retention factor, k, must be modified relative to conventional chromatography because of the mobility of the pseudo-stationary phase. The equation becomes [6]:

$$k = \frac{t_{mig} - t_0}{t_0 \cdot \left(1 - \frac{t_{mig}}{t_{mc}}\right)}$$

where t_{mig} is the migration time of the analyte, t_0 is the migration time of a neutral solute which does not interact with the micelles, and t_{mc} is the time required for a micelle to travel the length of the capillary. t_{mc} is usually measured using an analyte which spends all of its time associated with the micellar pseudo-stationary phase, but can be estimated using a homologous series and the reiterative approach of Bushey and Jorgenson [7].

As reviewed in this volume, conventional micelles are very useful as pseudo-stationary phases. Many commercial surfactants with varying selectivity are available to be employed as pseudo-stationary phases [8–10]. However, there are significant limitations associated with MEKC and with conventional micelles which have led many researchers to seek alternative pseudo-stationary phases.

MEKC is characterized by a limited migration time range: all uncharged analytes must have migration times between t_0 and t_{mc}. Hydrophobic analytes present problems because, due to high partition coefficients, they tend to have migration times close to t_{mc} with very high retention factors. Due to this limited migration range, optimization of separations requires that the retention factors be adjusted to a limited range [11]. Adjustment of retention factors to this limited range is not always possible with conventional micelles due to limitations of the micelles themselves.

Commercial surfactants have not been developed with chromatographic selectivity in mind. Chiral separations by MEKC have thus required the addition of chiral modifiers [12–14] or the synthesis [15] and application of chiral pseudo-stationary phases [16–23]. There is substantial evidence that the surfactants used for MEKC interact with chiral modifiers such as cyclodextrins [24–27], which may limit the chiral selectivity.

Conventional micellar pseudo-stationary phases have limited stability, being in a state of equilibrium with the free surfactant in the surrounding buffer medium. The micellar equilibrium is characterized by the critical micelle concentration (CMC) of free surfactant and the aggregation number, or the number of surfactant monomers assembled in a single micelle. Since the micelle is the pseudo-stationary phase in MEKC, the retention factor is directly related to the volume of the micelle, V_{mc}, through

$$k = K\left(\frac{V_{mc}}{V_{aq}}\right)$$

where K is the distribution coefficient and V_{aq} is the volume of the aqueous phase excluding the volume of the micelle. The volume of the micelle is given as

$$V_{mc} = \bar{\nu}(C_{srf} - \mathrm{CMC})$$

where $\bar{\nu}$ is the partial specific volume of the surfactant and C_{srf} is the total concentration of the surfactant. The CMC varies with the analytical conditions: it is affected by changes in the temperature, salt concentration, pH and the concentration and nature of buffer additives.

The dependence of the CMC on the temperature is particularly problematic since the application of the electric field across the capillary causes Joule heating and a rise of the temperature inside the capillary, even when it is thermostatted [28–31]. A change in the temperature will cause a change in the CMC, the distribution coefficient and the viscosity of the buffer. Because of the dependence of the retention factor on the CMC and the distribution coefficient, temperature effects can be expected to be more serious in MEKC than in CE.

Another serious impact of the equilibrium status of the micellar phase is that it limits the flexibility of the technique in terms of the choice of analytical conditions. The surfactants must have a relatively

low CMC, limiting the choice of surfactants considerably. The surfactant concentration must be high relative to the CMC, or irreproducibility will result. Additionally, the effect of organic additives on the CMC and structure of micelles adds complications for the analysis of hydrophobic compounds.

Finally, the use of conventional surfactants limits the applicability of MEKC for mass spectrometric detection. Unless the surfactant is somehow removed, the presence of a high concentration of low-molecular-mass surfactant leads to large signals in the low-molecular-mass region of the mass spectra, interfering with most MEKC analyses.

An ideal pseudo-stationary phase would have several properties. To provide desired chromatographic selectivity, phases should be available with a wide range of chemical structures. To permit reproducibility and adjustment of the retention factor, they should be stable with respect to changes in the analytical conditions and be stable and soluble in the presence or absence of organic modifiers. To provide a wide migration range, they should have high electrophoretic mobility. To permit application of secondary media such as cyclodextrins and to minimize Joule heating they should have very low or zero CMC. High-molecular-mass would permit mass spectrometric detection. To provide efficient separations, they should be monodisperse and provide fast mass transfer for analytes between the pseudo-stationary phase and buffer medium. Conventional micelles, while they are useful for many routine applications of MEKC, will never meet all of these criteria. This has led to the development and evaluation of alternative pseudo-stationary phases.

Several types of alternative pseudo-stationary phases have been employed. Neutral pseudo-phases have been employed to provide selectivity for ionic compounds. Cyclodextrin polymers [32–34] have been used to provide chiral selectivity, and polyvinylpyrrolidone [35–40] to separate diastereomeric derivatives of enantiomers. Proteins [41–44] and charged cyclodextrins [45–50] have also been employed for chiral separations. Resorcarenes [51] are stable structures that permit the separation of hydrophobic compounds, but which are limited by background UV absorbance. Dendrimers [52–56] and modified dendrimers [57] have also been utilized as monomolecular pseudo-stationary phases.

Several authors have reported the use of covalently stabilized high-molecular-mass surfactants or micelle polymers as pseudo-stationary phases in MEKC [10,58–72]. These are amphophilic polymers with both hydrophilic and hydrophobic regions. Because these polymers have similar properties to conventional surfactant micelles (solubilization, surface active properties, structure determined by hydrophobic effect), they are often referred to as polysoaps or micelle polymers. The fundamental difference between micelle polymers and conventional micelles is that in the polymer the "micelle" size and structure is fixed by covalent bonds, rather than by hydrophobic association and self-assembly. Two recent reviews have covered the origins, structure, and properties of micelle polymers and polymer surfactants in great detail [73,74]. In this review, the term micelle polymers will be used to refer to polymers synthesized in micellar form, and the term high-molecular-mass surfactants will be used to refer to other amphophilic polymers.

Applied in MEKC, these compounds provide very stable pseudo-stationary phases with zero CMC. Because the CMC is zero, the phases can be used at virtually any concentration. The structure and concentration of the phase does not change with changes in the analytical conditions. The structures can be used in the presence of relatively high amounts of organic modifier [54,58,59,62,63,66,67,71], with mass spectrometric detection [64], and often afford unique selectivity relative to micelles of SDS [10,58,59,67,71]. Chiral separations can be achieved by using cyclodextrins [63] or by using chiral polymers [61,65,68–70]. Micelle polymers and dendrimers are may have advantages over other types of polymer surfactants in that they are relatively monodisperse. In principle, polymers could be synthesized and employed with virtually any selectivity or electrophoretic mobility, since the requirement of self-association is eliminated. In short, these compounds have the potential to provide many of the ideal properties of a pseudo-stationary phase mentioned above. The exceptions are that they may not be monodisperse, and the mass transfer kinetics may be slower than with conventional micelles. Both of these limitations would lead to diminished plate counts relative to conventional micelles.

In MEKC application, these structures have been

given many names: polymer micelles, high-molecular-mass surfactants, polymer surfactants, molecular micelles, and monomolecular pseudo-stationary phases. Because of confusion with micelles formed from the polymers, the term polymer micelles should be avoided [73]. Micelle polymers are macromolecules synthesized in micellar form, while polymer surfactants are any polymers with surfactant properties. This review concentrates on the use of polymer surfactants, micelle polymers and dendrimers as pseudo-stationary phases. Fig. 1 shows the structures of the phases to be reviewed. The review is organized by the structures of the polymers. Those readers interested in chiral separations are referred to the sections on poly(sodium-undecenoyl valine) and acrylate polymers. Those readers interested in the use of micelle polymers for MEKC–MS application should refer to Section 4.

2. Initial reports

The first report of an ionic polymer as a pseudo-stationary phase was that of Wallingford and Ewing [75]. As a brief note, they reported the use of monodisperse polymer particles as a pseudo-stationary phase. The polymer, Eastman AQ-55, is a copolymer of isophthallic acid, isophthallic acid-5-

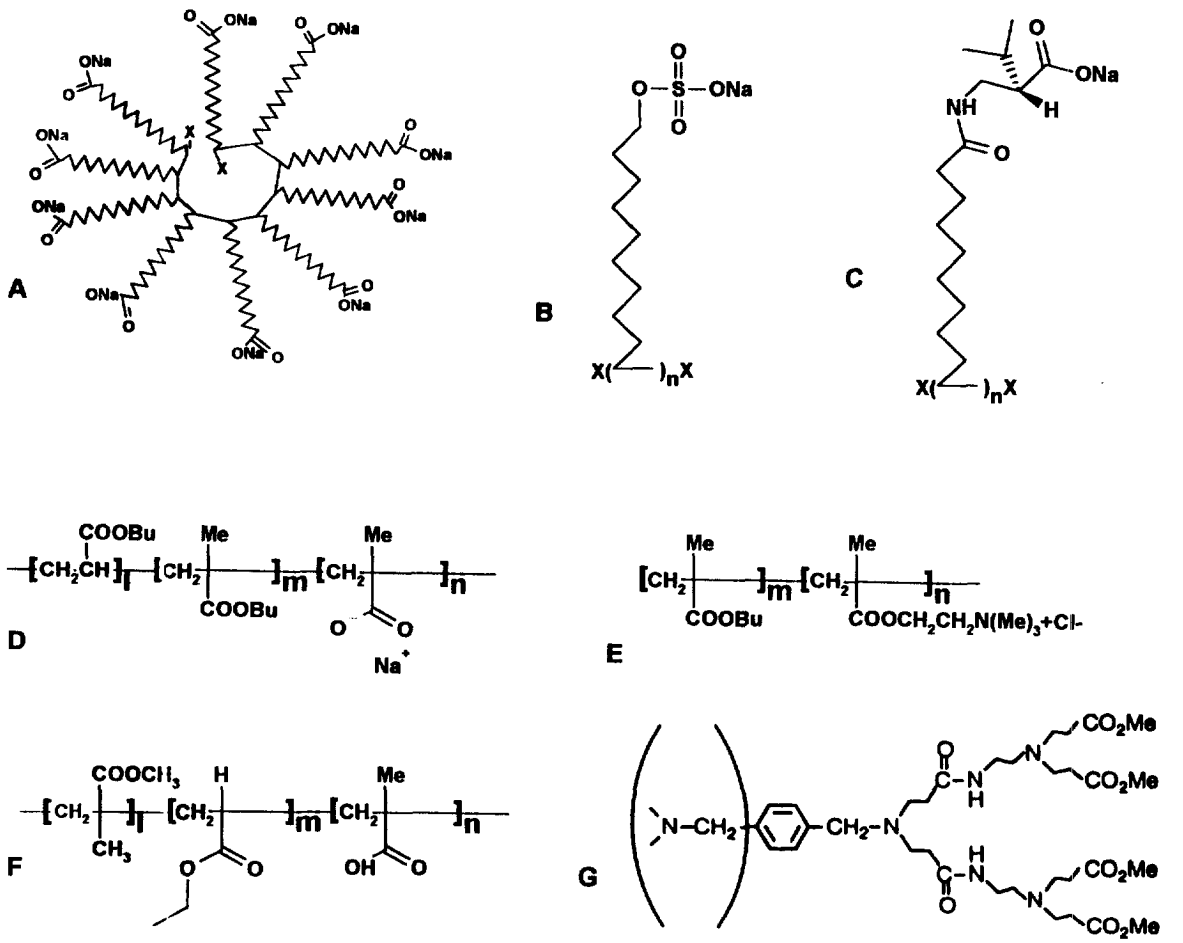

Fig. 1. Structures of the micelle polymers (A–C) and polymer surfactants (D–F) employed to date. Also illustrated is an example of dendrimer chemistry (G) employed as a pseudo-stationary phase [54].

sulfonate, 1,4-cyclohexane dimethanol and diethylene glycol. In aqueous solution, the polymer forms a monodisperse suspension of particles 26 nm in diameter. The particles have a lipophilic interior and an ionic exterior. The polymer is a strong absorber in the UV region. Wallingford and Ewing employed electrochemical detection. They reported that among the potential advantages of polymeric pseudo-stationary phases is their stability in the presence of organic modifiers. However, they expressed concern about the kinetics of the interactions with such phases and the resulting plate numbers.

This author also has experience with these polymers [76]. NBD-amines were separated and detected by fluorescence detection. Attempts were also made to separate polynuclear aromatic hydrocarbons (PAHs) and detect them by UV absorption at wavelengths above 300 nm. A separation of NBD-amines is shown in Fig. 2. The migration order is abnormal, and the plate number varied for a single separation from 4500 to 90 000 plates. Additionally, no separation was achieved for PAHs in 40% methanol. All of the PAHs migrated with t_{mc}, indicating that the polymer is extremely hydrophobic in methanolic solutions. This may be due to absorption of methanol into the polymer interior. In 20% acetonitrile the polymer was found to have a broad range of electrophoretic mobilities.

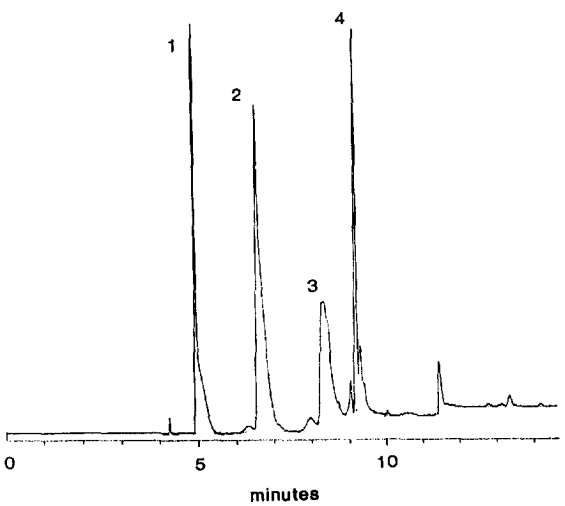

Fig. 2. Separation of selected NBD-amines using Eastman AQ-55 as a pseudo-stationary phase. Peaks: 1 = NBD-*n*-pentylamine; 2 = NBD-*n*-heptylamine; 3 = NBD-aminofluorene; 4 = NBD-*n*-propylamine. Conditions: 57 cm (50 cm effective length) × 50 μm Beckman fused-silica capillary, 30 kV applied potential, Beckman P/ACE LIF detector, ex. 488 nm, em. 520 nm; running solution 60% 0.10 *M* sodium borate, pH 9, 40% methanol, 0.1% (w/v) Eastman AQ-55; analyte concentration 8 ppm.

In two separate early reports [77,78], Terabe and Isemura used a cationic polymer as a pseudo-stationary phase for the separation of isomeric ions having identical electrophoretic mobilities. The polymer employed was not a micelle polymer, and the separation was achieved via an ion-exchange mechanism.

3. Micelle polymers

3.1. Poly(sodium 10-undecylenate)

The first successful reports of the use of a micelle polymer in the open literature were those of Palmer and coworkers [58,59]. These authors used a true micelle polymer of sodium-10-undecylenate (SUA, Fig. 1A) as a pseudo-stationary phase to achieve the separation of alkyl phthalates and PAH in buffers modified with up to 50% methanol or 45% acetonitrile. The authors indicated that the stability of the polymer, which permitted the use of organic modifiers for the separation of hydrophobic compounds, was its greatest advantage.

The polymerization of sodium undecylenate had been studied in some detail [79–86]. Sodium 10-undecylenate can be polymerized by gamma irradiation, UV irradiation, or free radical initiation, but only above the CMC. The surfactant evidently undergoes reaction at the tail end of the lipid chain in the interior of the micelle to form an oligomer of ten monomer units. Solutions of the resulting oligomer behave similarly to micellar solutions of the free surfactant. Organic molecules are solubilized by the oligomer in a similar fashion to micelles of the free surfactant, although spectroscopic studies have shown that the solubilized molecules are in a more rigid and polar environment [79]. There is some evidence that the polymer forms intermolecular micelles at concentrations as low as 0.0002 *M* [85]. A recent study indicates that the conformation of the micelle polymer changes at pH values above 8.5 to yield a more open and hydrophobic structure [86].

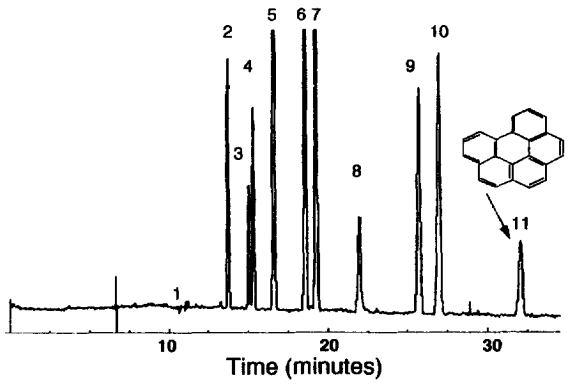

Fig. 3. Separation of PAHs using poly(sodium undecylenate) as a pseudo-stationary phase. Peaks: 1 = methanol; 2 = naphthalene; 3 = acenaphthene; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = pyrene; 8 = chrysene; 9 = benzo[*b*]fluoranthene; 10 = benzo[*a*]pyrene; 11 = benzo[*g,h,i*]perylene. Conditions: 50 cm effective length × 100 μm fused-silica capillary, applied potential 200 V/cm, solution 1% poly(sodium undecylenate) in acetonitrile–aqueous borate (35:65) buffer (0.012 *M*, pH 8.2); UV detection at 275 nm. Reprinted from [59] with permission from Hüthig.

As a pseudo-stationary phase the polymer performs very well. Fig. 3 shows a separation of PAHs in 35% acetonitrile. The polymer provides efficient and selective separations of hydrophobic compounds. The electrophoretic mobility of the polymer increases substantially between 30% and 40% acetonitrile, providing a broad elution range. Additionally, the plots of log k vs. percent acetonitrile were non-linear. This increase in mobility and apparent change in interaction were interpreted to mean that the structure and solvation of the polymer are dynamic. Also reported was that the selectivity of the polymer is significantly different from that of SDS micelles. The polymer was observed to be more retentive of polar compounds, and less retentive of non-polar compounds.

Problems were also reported. The anodic buffer became cloudy after several runs, and the migration times and retention factors of the PAHs were observed to increase over time. The former was attributed to the carboxylic acid head groups and hydrolysis of the anodic buffer, and the latter to evaporation of the organic modifier.

Still, these studies demonstrated conclusively the utility of micelle polymers as pseudo-stationary phases, and led to the development and characterization of similar materials.

3.2. Poly(sodium 10-undecenylsulfate)

In part to eliminate the problems with the carboxylate head group, and in part to prepare a micelle polymer with similar chemistry to SDS, Palmer and Terabe synthesized and employed the sulfate analog of sodium undecylenate, sodium undecenyl sulfate (SUS, Fig. 1B) [62,67,71]. Like its carboxylate counterpart, this polymer provided efficient and selective separations of a variety of compounds in aqueous and modified aqueous buffers. The polymer was also employed for the separation of a series of substituted benzene and naphthalene compounds. A comparison of the separation achieved with the sulfate polymer and SDS micelles is shown in Fig. 4. Changes in migration order indicate the different selectivity of the polymer and the SDS micelles.

Shamsi et al. have also utilized this polymer for the separation of PAHs and with cyclodextrins for chiral separations [87].

In a more detailed study of the selectivity of the undecylenate and undecenyl sulfate polymers relative to each other and to SDS, Palmer and Terabe have shown that the two polymers are very similar in selectivity [71]. Both polymers interact more strongly with compounds having amine or hydroxyl groups, implying that they are more polar or are better hydrogen bond acceptors. The actual reason for the stronger interactions with more polar compounds is not well understood. It is possible that this is simply a result of the smaller size of the SUA and SUS polymers relative to SDS micelles. It was also thought that polymer terminating groups on the interior of the micelle polymer might provide a more polar interior. However, recent results in the authors laboratory using hydrophobic initiators have indicated that this is not a significant factor.

The SUS polymer is also useful in buffers modified with organic solvents. Relative to micelles of SDS, the SUS polymer retains greater electrophoretic mobility and greater selectivity in buffers modified with acetonitrile and methanol [71]. Figs. 5 and 6 show the separation of twelve PAHs in methanol- and acetonitrile-modified buffers, respectively using SDS and the SUS polymer. It can be seen that the

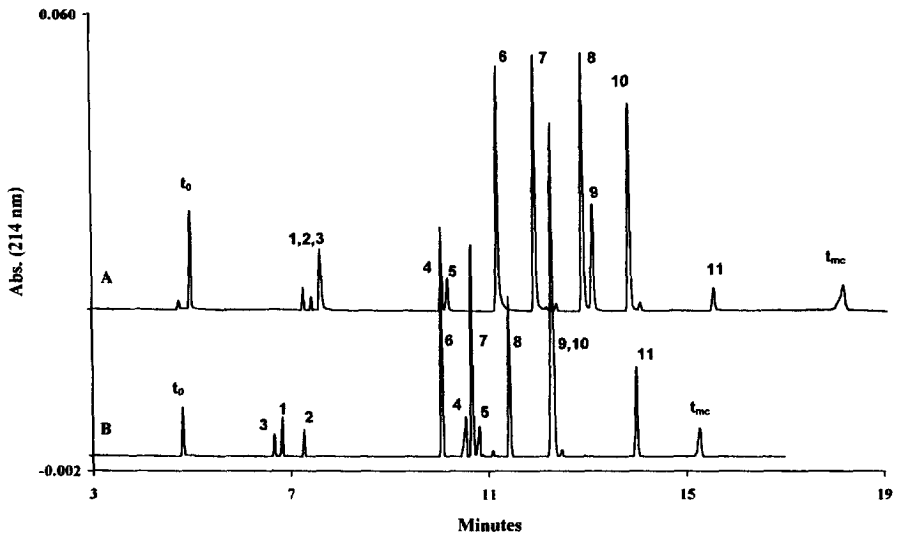

Fig. 4. Separation of substituted benzene and naphthalene compounds using poly(sodium undecenyl sulfate) and SDS as pseudo-stationary phases. Peaks: 1 = nitrobenzene; 2 = anisole; 3 = *p*-nitroaniline; 4 = *o*- xylene; 5 = *m*-xylene; 6 = naphthylamine; 7 = naphthalene methanol; 8 = acenaphthenol; 9 = naphthalene; 10 = naphthalene ethanol; 11 = diphenyl ether. Conditions: 57 cm (50 cm effective length) × 50 μm capillary; running solutions (A) 0.83% SUS polymer; (B) 30 m*M* SDS in phosphate–borate buffer at pH 7.3; UV absorbance detection at 214 nm. Reprinted with permission from [71], copyright 1997, American Chemical Society.

polymeric phase provides much better separations due to the greater migration time range. The structural selectivity of the polymer vs. SDS micelles has also been studied. In 60% methanol the structurally rigid PAHs (rings connected at more than one point) have less affinity for the polymer phase relative to SDS micelles, while more flexible compounds (rings connected by a bridging bond) are more highly attracted to the polymer phase. This is understandable given the smaller size and more rigid structure

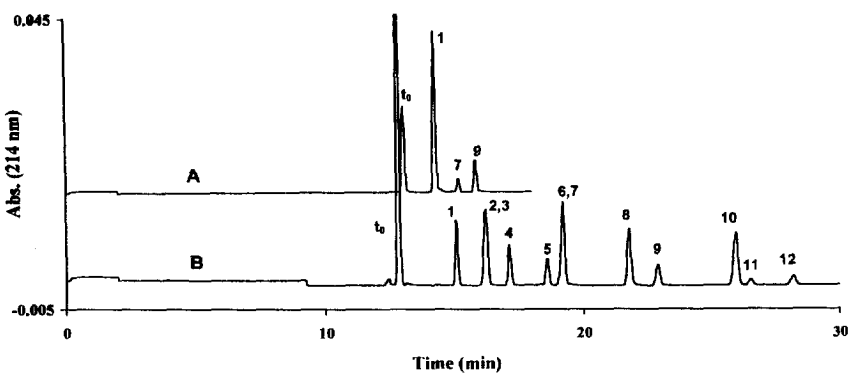

Fig. 5. Separations of PAHs in 60% methanol using SDS and poly(sodium undecenyl sulfate). Peaks: 1 = naphthalene; 2 = diphenyl; 3 = diphenylmethane; 4 = fluorene; 5 = phenanthrene; 6 = *o*-terphenyl; 7 = anthracene; 8 = fluoranthene; 9 = pyrene; 10 = *m*-terphenyl; 11 = *p*-terphenyl; 12 = triphenylene. Only analytes 1, 7 and 9 were injected for the SDS separation. Conditions: 47 cm (40 cm effective length) × 50 μm capillary; applied potential 18.5 kV; running solutions A 0.02 *M* SDS; B 0.6% (w/v) SUS in methanol–pH 9.3 borate (60:40) buffer. Reprinted with permission from [71], copyright 1997, American Chemical Society.

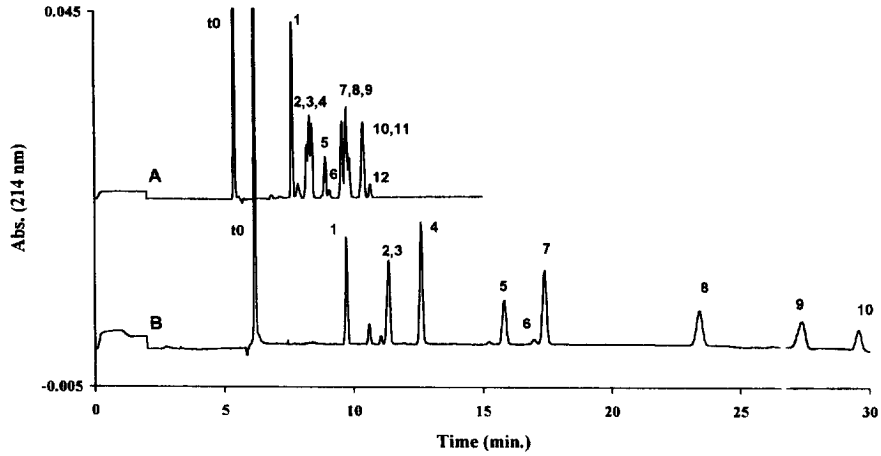

Fig. 6. Separations of PAHs in acetonitrile–pH 9.3 borate (40:60) buffer. (A) 50 m*M* SDS. (B) 1% (w/v) SUS polymer. Both separations were conducted in 37 cm (30 cm effective length) capillaries with an applied potential of 13 kV. Analytes are identified in Fig. 5. Reprinted with permission from [71], copyright 1997, American Chemical Society.

of the polymer which may render it less able to accommodate large inflexible molecules. However, in 40% acetonitrile the opposite result is observed. The more rigid compounds, with the exception of the very large and inflexible triphenylene, have greater attraction for the SUS polymer relative to the SDS micelles. This result is more difficult to understand, and implies that there are significant differences in micellar and/or polymer structure in methanol- vs. acetonitrile-modified buffers.

A significant problem with the use of SUA and SUS polymers is the synthesis. These polymers are not commercially available, and the synthetic yield for the polymerization by free radical initiation is rather low. Palmer and Terabe also report that the original purification method is not sufficient, leaving the product contaminated with sodium sulfate [71]. Further purification by dialysis or an alternative method is required to obtain pure polymer. These problems are presumably avoided if UV or gamma irradiation are employed to induce polymerization.

3.3. Poly (sodium undecenoyl-L-valinate)

One of the limitations associated with conventional micelles is the lack of chiral selectivity. Chiral surfactants have been synthesized and employed for chiral separations [15–23]. Wang and Warner [61,68], Dobashi et al. [65], Agnew-Heard et al. [69] and Williams et al. [70] have reported modification of sodium undecylenate with L-valine to obtain a chiral surfactant. The surfactant was then polymerized by γ [61,68–70] or UV [65] irradiation to obtain the chiral micelle polymer (Fig. 1C).

Two 1992 Japanese patents [88,89] show the separation of chiral compounds using two chiral micelle polymers. Poly(sodium undecenoyl-L-valine) and a cationic amide of this compound were synthesized, polymerized and employed for chiral separations. The two structures were patented for use in chiral separations by electrokinetic chromatography.

Wang and Warner first reported the use of this polymerized chiral micelle in the open literature in 1994 [61]. Substantially improved separations of (±)-1,1′-bi-2-naphthol were observed when poly(sodium undecenoyl-L-valine) was employed relative to the monomer surfactant. The migration order of the analytes was reversed when poly(sodium-undecenoyl-*R*-valine) was employed as a pseudo-stationary phase. The elimination of the dynamic equilibrium associated with conventional micelles was the explanation given for better separations obtained with the polymerized micelle. Better plate counts were observed with the chiral micelle which the authors explained as being due to a more compact structure of the polymerized micelle leading to faster mass transfer kinetics. However, it seems possible that the lower efficiency in the conventional micellar

case was due to Joule heating caused by the relatively high critical micelle concentration of the monomer surfactant. The authors report better separations at pH 10 than at pH 9, which they attribute to a more open structure of the polymer at high pH [86] which leads to better interactions.

Dobashi et al. concentrated on the separation of dinitro benzoyl amino acid isopropyl esters and compared the separations achieved with micelles formed from chiral surfactants to those obtained with the chiral micelle polymer [65]. As with Wang and Warner, the reasoning for employing a micelle polymer was that the association–dissociation of conventional micelles may determine the degree of chiral recognition. A separation of derivatized amino acids using the chiral micelle polymer is shown in Fig. 7. Using the polymer, however, the selectivities

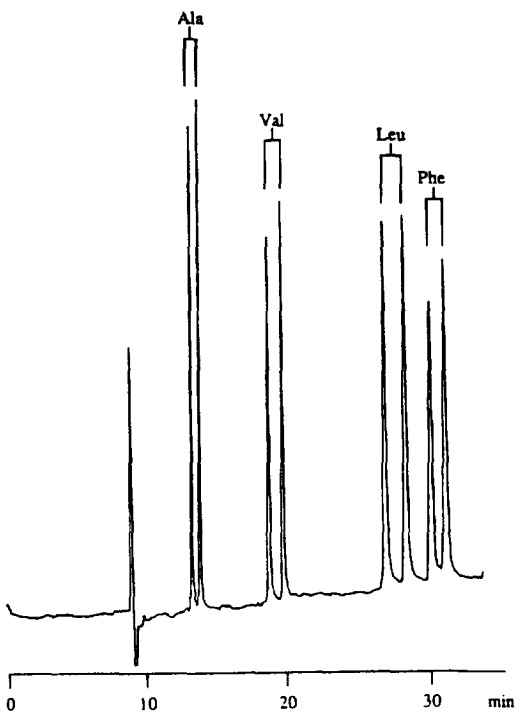

Fig. 7. Separation of a mixture of four enantiomeric (3,5-dinitrobenzoyl)amino acid isopropyl esters with poly(sodium undecenoyl-L-valinate) solution by electrokinetic chromatography. 50cm×50 μm I.D. fused-silica capillary, applied potential 12.4–12.8 kV, UV detection at 254 nm. 0.76% poly(sodium undecenoyl-L-valinate) in 0.025 *M* borate–0.05 *M* phosphate buffer (pH 7.0) containing 2 *M* urea and 0.01 *M* SDS. Reprinted with permission from [65], copyright 1995, American Chemical Society.

were not as good as those obtained when conventional micelles of sodium dodecanoyl-L-valine) were employed. Additionally, peak tailing could only be eliminated by the addition of sodium dodecyl sulfate to the separation buffer. They conclude that the increased order of the polymer relative to the micelles does not prevent binding of the substrate molecules and that an ordered interfacial region where enantiomer binding and recognition can occur exists in either case. The lower selectivity observed with the micelle polymer was attributed to spaces between the surfactant monomers and penetration of water into the interior of the micelle polymer.

In further studies with the same polymer [68–70], the research group of Professor Warner at Louisiana State University has shown that poly(sodium undecenyl-L-valinate) can be employed successfully for a variety of chiral separations.

The polymer has been employed in combination with γ-cyclodextrin [68]. The combined effect of the chiral polymer and the chiral recognition of the cyclodextrin provided greatly improved separations of (±)1,1′-binaphthol, (±)verapamil, (±)binaphthyl diyl hydrogen phosphate and D,L-laudanosine. Chiral resolution of 2.5 to 6.5 was reported. The micelle polymer, because of its greater size, can not be included in the CD cavity and does not interfere with the chiral recognition of the cyclodextrin. It is also shown that an advantage of the chiral micelle polymer is that it can be employed in buffer media modified with organic solvents. In some cases, the addition of methanol up to 40% did improve the chiral separations, but addition of acetonitrile had a detrimental effect on the separations.

Employed without cyclodextrins, the polymer has been shown to provide selectivity for Trögans base, binaphthyl, paveroline and coumarin derivatives [69]. Each of the separations required substantial optimization of pH and polymer concentration. Anionic analytes do not interact strongly with the anionic micelle polymer, but can be separated. Interaction with the core of the micelle polymer through hydrophobic interactions did not contribute to chiral selectivity. The micelle polymer was found to precipitate at pH values below 5.5. A polyvinyl alcohol coated capillary was employed to eliminate any adsorption of the micelle polymer on the surface of the capillary. This improved the separation for

two of the paveroline derivatives, but eliminated all selectivity for the third.

In a recent review, the same group concentrates on their results with poly(sodium undecenoyl-L-valinate) [70]. They present an argument for the fundamental advantage of chiral micelle polymers over conventional chiral micelles based on the complications of multiple equilibria and the polydispersity of conventional micelles. There is no general concensus, however, concerning the polydispersity of micelles [90]. If micelles are polydisperse, the same might be argued for the micelle polymers, since they are synthesized in micellar form. In addition, polydispersity is not thought to contribute substantially to band broadening in MEKC. The authors do not present any further experimental validation of their assertion that the micelle polymer is superior with comparative studies.

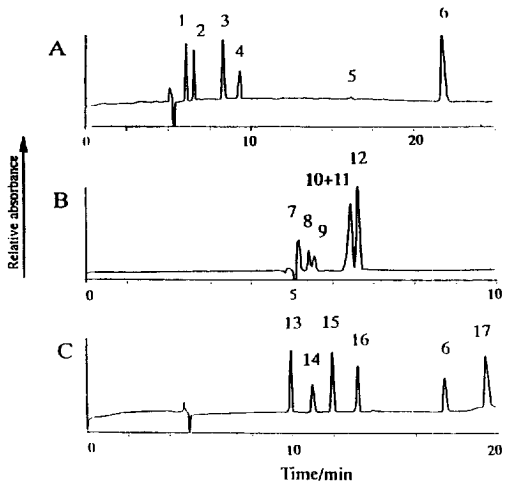

Fig. 8. MEKC separations of benzene derivatives (A), cold medicines (B) and naphthalene derivatives (C) using BBMA as a pseudo-stationary phase. Peaks: 1=resorcinol; 2=phenol; 3=*p*-nitroaniline; 4=nitrobenzene; 5=toluene; 6=2-naphthol; 7=acetaminophen; 8=caffeine; 9=guafenesin; 10=ethenzamide; 11=isopropylantipyrine; 12=trimetoquinol; 13=1-naphthalene methanol; 14=1,6-dihydroxynaphthalene; 15=1-naphthylamine; 16=1-naphthalene ethanol; 17=1-naphthol. Conditions: 36.5 cm (32 cm effective length)×50 μm capillary; running solution 2% unpurified BBMA in 50 m*M* phosphate–100 m*M* borate buffer (pH 8.0); applied potential 20 kV; detection wavelength 210 nm. Reprinted with permission from J. Chromatogr. [60].

4. Acrylate copolymers

Polymer surfactants with the forms shown in Fig. 1D–F have been employed for MEKC separations of cold medicine ingredients [41,60], substituted benzenes [10,41,60], substituted napthalenes [41,60] and hydrophobic compounds (PAHs, *n*-alkyl phenones, fullerenes) [66]. The polymers have also been employed with cyclodextrins for the separation of dansyl amino acids [63] and for MEKC with mass spectrometric detection [64]. The chemical selectivity of these polymers has also been studied in some detail [10].

Terabe et al. [41] and Ozaki et al. [60] were the first to report the used of an acrylate copolymer as a pseudo-stationary phase. They employed butyl acrylate-butyl methacrylate-methacrylic acid (Fig. 1D, BBMA) copolymers for the separation of benzene derivatives, cold medicine ingredients and naphthalene derivatives, as shown in Fig. 8. In comparison with SDS micelles, BBMA had similar selectivity for the cold medicine ingredients and benzene derivatives, but significantly different selectivity for the substituted naphthalene compounds. 1-Naphthol migrated much more slowly than naphthalene methanol or naphthalene ethanol, which was not observed with SDS micelles. The separation of cold medicine ingredients was unsuccessful because the retention factors were too small. This was attributed to the presence of carboxylate groups on the BBMA. The effects of pH and polymer structure were also studied. At pH values below 4 the BBMA was insoluble due to lack of surface charge. From pH 4 to 7 the migration range expanded considerably due to increased ionization of the carboxylate groups. At the same time, the retention factors for naphthalene compounds decreased, also due to increases in surface charge. Increases in the fraction of methacrylic acid in the copolymer had similar effects: at higher fractions where the surface charge is greater the elution range was greater and the retention factors were lower. It was concluded by the authors that changes in the polymer chemistry are more favorable than changes in the pH, since pHs below 4 cause precipitation and since changes in pH are accompanied by changes in the electroosmotic mobility. Significantly, three different molecular masses of the BBMA polymer gave essentially the

same separations of naphthalene derivatives. As long as the polymer chemistry was constant, the molecular mass did not affect the electrophoretic mobility of the polymer or the retention factors of the solutes. This led the authors to conclude that dispersity in the polymer molecular mass should not lead to diminished plate counts.

In further work with BBMA Ozaki et al. [63] investigated the effects of the addition of methanol and a nonionic surfactant, octaoxyethylenedodecanol [$(EO)_8R_{12}$]. Addition of methanol to the run buffer was found to reduce the retention factors of substituted naphthalene compounds, as would be expected from reductions in hydrophobic interactions. Minor selectivity changes were also noted. Similar results were observed with SDS micelles. Addition of $(EO)_8R_{12}$ was found to increase the retention factors of the same compounds, while the migration range was diminished. This indicates that the nonionic surfactant forms comicelles with the polysoap, rather than forming independent nonionic micelles. If independent micelles had been formed, the retention factors would have been reduced by competitive partitioning into the nonionic micelles.

In the same paper Ozaki et al. also demonstrated the utility of BBMA in combination with cyclodextrins for the chiral separation of dansylated amino acids [63]. In combination with 10 m*M* β-CD nine of ten pairs of dansylated amino acids were successfully separated, and eight had separation factors greater than those observed with SDS and 60 m*M* β-CD. Seven of the ten were separated when γ-CD was employed with BBMA, but none were separated when α-CD was employed. The results clearly show that BBMA is superior to SDS for the chiral separation of dansylated amino acids. This can be attributed to the absence of monomeric surfactant molecules in the BBMA case. Monomeric surfactant molecules can be co-included in the cyclodextrins [24–27], reducing chiral selectivity. The BBMA can not be co-included in the cyclodextrin cavity, owing to its large size. It was also demonstrated that it is important to purify the polymer of low-molecular-mass impurities, as these impurities can also interfere with the separation.

Also demonstrated in this paper was the use of a cationic acrylate copolymer, butyl methacrylate-methacryloyloxyethyltrimethylammonium chloride copolymer (BMAC, Fig. 1E) [63]. BMAC was not soluble in an aqueous buffer without 20% 2-propanol. The direction of electroosmotic flow was reversed due to adsorption of the cationic polymer on the capillary walls. The plate counts were just as high with BMAC as with BBMA, indicating that adsorption onto the capillary walls does not limit the efficiency. The migration order of substituted naphthalene compounds was found to be similar to that with BBMA, but significantly different from that with SDS micelles.

In a third paper, Ozaki et al. have demonstrated that BBMA can be used as a pseudo-stationary phase for the combination of MEKC with mass spectrometric (MS) detection [64]. Fig. 9 illustrates the concept of the use of polysoaps or micelle polymers as pseudo-stationary phases for MEKC–MS with an electrospray interface. A conventional surfactant will dissociate during the electrospray process, and the monomeric surfactant molecules may be ionized and cause an interference in the mass spectrum, or may inhibit ionization of the analytes. A polymeric

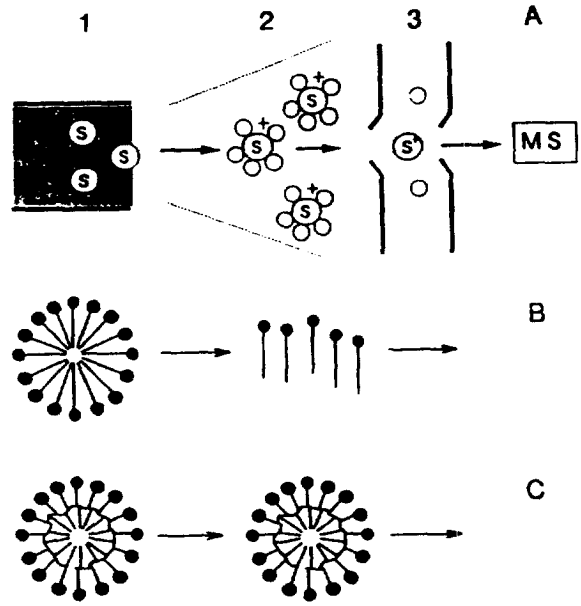

Fig. 9. (A) Schematic illustration of an electrospray interface and expected behaviour of (B) low-molecular-mass and (C) high-molecular-mass surfactant micelles. 1 = Capillary; 2 = electrospray; 3 = mass spectrometer; S = solute molecule; S^+ = solute molecular ion; O = solvent. Reprinted with permission from J. Chromatogr. [64].

pseudo-stationary phase, on the other hand, will not dissociate in the electrospray, and if ionized will not interfere in the low-molecular-mass region of the mass spectrum where MEKC analytes would be most likely to give signals. An additional advantage of polymeric pseudo-stationary phases for MS applications is that they can be employed at low concentrations without the need to maintain a critical micelle concentration. The results in this paper are somewhat preliminary, but are significant. BBMA was shown to provide a clean mass spectrum from 50 to 1000 m/z, while SDS micelles had strong interferences at 310 and 610 m/z. An interference at 310 m/z with BBMA was attributed to a stabilizer which had been added to the polymer. 1-Naphthyl amine, quinine sulfate, tetraphenylphosphonium chloride and octaoxyethylenedodecanol (as a micelle marker) were separated and detected by mass spectrometry using 2% BBMA as the pseudo-stationary phase. The results using MEKC–MS and CE–MS for these analytes are illustrated in Fig. 10. Introduction of 2% BBMA into the electrospray system generated a stable electrospray and did not impair MS detection significantly. The intensity of the signals for each of the analytes was reduced as the concentration of BBMA was increased from 0 to 2%. This was attributed to suppression of electrospray ionization consistent with that observed as a function of increases in salt concentration.

Yang et al. have used a similar polysoap, poly-(methyl methacrylate-ethyl acrylate-methacrylic acid) (Fig. 1F, Elvacite 2669) as a pseudo-stationary phase for the separation of hydrophobic compounds [66], and have used linear solvation energy relationships (LSER) to characterize the chemical selectivity of this polymer relative to several conventional micelles [10]. In their initial report, Elvacite 2669 was employed for the separation of PAHs, *n*-alkylphenones and fullerenes in buffers modified with high concentrations of organic solvents [66]. *n*-Alkylphenones with alkyl chains up to 24 carbons in length were separated in up to 70% methanol. Thirteen PAHs were separated in less than 30 min in 50% methanol. Separation of fullerenes C_{60} and C_{70} was achieved in a mixed solvent of 40% methanol, 20% acetonitrile and 16% 1-propanol. The efficiency and selectivity of the separations was good, although some peak tailing was observed. In the more recent

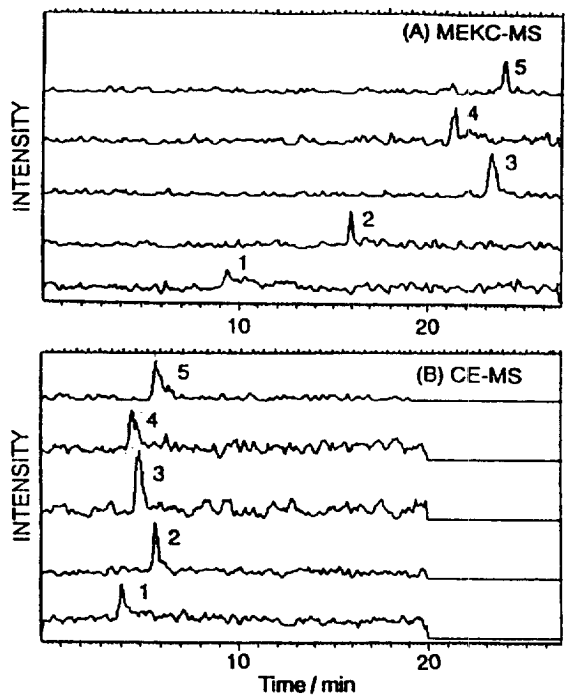

Fig. 10. Single-ion chromatograms obtained by (A) MEKC–ESI-MS and (B) CE–ESI-MS. Solutes: 1 = phenyltrimethylammonium chloride; 2 = 1-naphthylamine; 3 = quinine sulfate; 4 = tetraphenylphosphonium chloride; 5 = octaoxyethylenedodecanol. MEKC conditions: 50 cm × 50 μm capillary; separation solution A 2% BBMA in 10% methanol and 10 m*M* ammonium formate buffer (pH 7) and B 10 m*M* ammonium formate buffer (pH 7); applied potential 13 kV. MS conditions: electrospray voltage 3 kV; drift voltage 70 V; focussing voltage 140 V; resolution 55; sheath liquid flow, water–methanol–formic acid (50:50:1, v/v/v) at ca. 5 μl/min. Reprinted with permission from J. Chromatogr. [64].

paper, LSER studies using sixty aromatic test solutes provided the relative cohesiveness and hydrogen bond acceptor and donor strength [10]. The polymer was found to have cohesiveness between hydrocarbon micelles (less cohesive) and flourocarbon micelles (more cohesive). The polymer was found to have intermediate hydrogen bond donor strength, between SDS and tetradecyltrimethyl ammonium bromide micelles. The hydrogen bond acceptor strength was relatively high: greater than sodium cholate micelles, but less than tetradecyltrimethyl ammonium bromide micelles. In a demonstration of these properties, the migration behavior of the sixty test compounds was described in detail on Elvacite 2669 relative to SDS micelles. Hydrogen bond donor

compounds or strong dipolar compounds were found to interact more strongly with the strong hydrogen bond accepting Elvacite 2669, while hydrogen bond acceptor compounds were found to interact more strongly with the hydrogen bond donating SDS micelles. Also interesting is that Elvacite 2669 has intermediate properties relative to those of conventional micelles, meaning that substantially different selectivity does not result from polymerization. A possible exception to this is the cohesiveness, which is more similar to a fluorocarbon micelle than a hydrocarbon micelle. This means that retention factors for Elvacite 2669 do not increase as rapidly with molecular size as they do with hydrocarbon micelles. This might be expected from the more rigid covalent structure of the polysoap, which would require greater energy to rearrange to solubilize larger analytes.

The acrylate copolymer BBMA has also been used in studies of the reproducibility of separations performed with polymeric pseudo-stationary phases relative to conventional micelles [72]. It is thought that the elimination of the micelle equilibrium should provide better reproducibity, since the size and structure of the pseudo-stationary phase should not be affected by changes in the analytical conditions. To date, the results of these studies have been mixed. BBMA does provide better migration time reproducibility than micelles of SDS, but quantitative reproducibility is not as good. The problems with quantitiative reproducibility may be due to a less stable baseline with BBMA, which may be caused by impurities in the polymer.

5. Dendrimers

Dendrimers are highly branched polymers that are synthesized in stages (generations) from a core. The polymers are constructed generation by generation using multi step repetitive syntheses, resulting in macromolecules with well-defined branches, very specific molecular masses and uniform sizes. The polymers differ from linear polymers in that they do not have entangled chains and they do have numerous chain-ends that can be functionalized. Dendritic molecules can be constructed with discrete domains having different properties. They have been described as "unimolecular micelles" [91,92]. Unlike micelles, however, dendrimers become more sterically hindered toward the exterior of the molecule, and the interior may be hydrophobic or hydrophilic.

These macromolecules have many properties which make them desirable as pseudo-stationary phases. They are stable in buffers modified with organic media, they are monodisperse, and they can be synthesized with many different chemistries or modified to provide unique selectivity.

Tanaka et al. were the first to report the use of dendrimers as carriers in electrokinetic chromatography [52]. Small starburst dendrimers (polyamidoamines) of one to four generations were synthesized, and two-and-one-half-generation dendrimers in carboxylate form were prepared by hydrolysis. The dendrimers were employed for the separation of substituted benzene and naphthalene compounds in aqueous buffers and for the separation of PAH in buffers modified with methanol. The selectivity of the dendrimers was shown to be significantly different from that of SDS or cetyltrimethylammonium chloride micelles, and the retention factors were shown to be much smaller than those with the micelles. The binding of solutes was shown to increase with the size of the dendrimers. The separation of PAH could be manipulated by the addition of methanol in a manner similar to that of reversed-phase chromatography. Greater interaction with PAH was observed with half-generation dendrimers under alkaline conditions than under acidic conditions, due to dissociation of the internal amino groups. Full generation dendrimers were not a selective for the separation of PAH, but could be employed in 100% methanol (0.05% acetic acid).

Kuzdzal et al. employed amide-based cascade macromolecules with carboxylic acid terminus for the separation of alkyl parabens and Robitussen cold medicine ingredients with good selectivity and efficiency [53]. No organic modifier was required to separate alkyl parabens up to butyl paraben. Good separations were obtained at pH 10, but pH 8 and 6 did not provide useful separations due to increased cationic behavior and reduced electrophoretic mobility of the dendrimer. The migration times were observed to increase significantly with increased size of the dendrimer from first to third generations. The

third generation dendrimer provided less efficiency than lower generations.

Tanaka et al. studied the selectivity of poly(amidoamines) with ammonia and *p*-xylenediamine as starting materials for the separation of substituted benzene and naphthalene compounds and for aromatic hydrocarbons [54]. The selectivity was found to be significantly different from that of conventional micelles. The dendrimers were found to recognize the backbone structure (benzene vs. naphthalene) of the analytes, but not the functional groups. Unlike the results of Kuzdzal et al. [53], no selectivity was observed for alkyl benzenes using the dendrimers.

While these differences in selectivity are interesting, the authors conclude that the utility of these dendrimers is not great [54]. The hydrophilic nature of the dendrimers leads to minimal interaction with the solutes, and increasing the concentration of the dendrimers leads to high current and a noisy baseline. The efficiency of the dendrimer system was observed to be lower than conventional micellar systems, possibly because of slow mass transfer.

Modifying these dendrimers with alkyl chains enhances their utility [57]. Half generation poly(amidoamine) dendrimers, hydrolyzed to obtain carboxylic acid end groups, can then be alkylated to varying degrees with alkyl chains of varying length. There can be some variation in the extent of alkylation, leading to a range of structures with different electrophoretic mobility and hydrophobicity. Nonetheless, modified dendrimers provide high-performance, and can be used for the separation of hydrophobic compounds in a full range (0–90%) of methanol-modified buffers. Dendrimers modified with dodecyl chains show greater utility than those modified with octyl chains, due to greater recognition of analyte functionality and hydrophobicity. While unmodified dendrimers recognize the backbone structure of the analytes, modified dendrimers provide selectivity more like that of SDS micelles. The modified dendrimers retain greater recognition of backbone structure, and thus provide unique selectivity relative to SDS micelles. Fig. 11 shows the separation of alkyl-phenyl ketones in methanol-modified buffers using SDS micelles and dodecyl-modified dendrimer. The SDS system shows an abnormal elution profile in 40% methanol, and very narrow migration windows above 40% methanol, while the dodecyl-modified dendrimer shows consistent separation up to 80% methanol. PAHs were also separated using the dodecyl derivative in 90% methanol. These results demonstrate the utility of dendrimers as backbone support for the synthesis of pseudo-stationary phases with a wide range of chemical selectivity.

Muijselaar et al. have studied the selectivity of a diaminobutane-based poly(propylenimine) dendrimer for the separation of substituted benzene compounds [55,56]. The dendrimer was found to have substantially different selectivity relative to micelles of SDS. The differences in selectivity are explained by the greater hydrogen bond accepting capabilities of the tertiary amines in the dendrimer, which led to greater interaction with hydrogen bond donating compounds such as hydroquinone and resorcinol.

The use of dendrimers as pseudo-stationary phases has only begun to be investigated. The ability to synthesize dendrimers with unique selectivity, or to modify dendrimers to provide desired selectivity, is an exciting development which should lead to significant improvements in pseudo-stationary phase technology in the coming years.

6. Comparison with capillary electrochromatography

Because of similar applications, the use of micelle polymers in electrokinetic chromatography begs comparison with capillary electrochromatography (CEC) [93–99]. In CEC capillaries are packed with modified silica particles, the stationary phase is covalently bonded to the particle surface, and the mobile phase is carried through the column by electroosmosis. Both techniques use stable phases for the separation of a variety of analytes. The stability of the phases permits the analysis of hydrophobic compounds and the use of mass spectrometric detection.

Micelle polymers have several potential advantages. They can be synthesized with a wide range of selectivities, and stored on the shelf until needed. Changing the pseudo-stationary phase requires only that the capillary be flushed and equilibrated with a new solution. Packed capillaries, with their various limitations, are not necessary. There are few, if any,

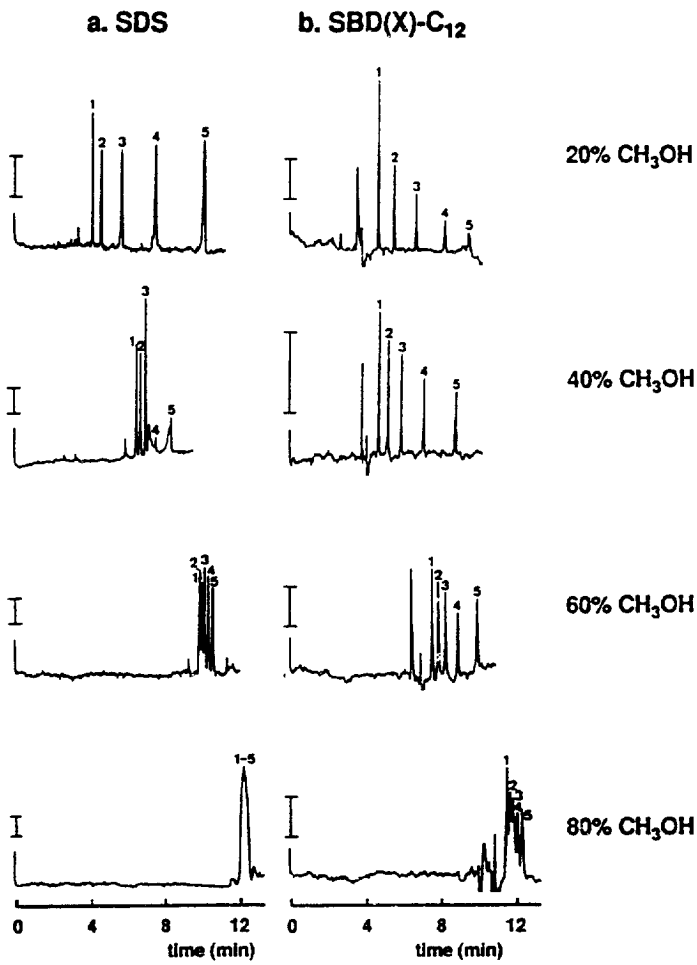

Fig. 11. Effect of methanol addition on the separation of alkyl phenyl ketones at 20–80% methanol. Solutes: alkyl phenyl ketones, C_6H_5-CO-C_nH_{2n+1} ($n=1$–5). Conditions: (a) applied potential 400 V/cm, 30 m*M* SDS, 20 m*M* borate, pH 9.3 in 20% methanol, pH 9.3 in 40% methanol, pH 9.0 in 60% methanol, pH 9.5 in 80% methanol; (b) 5 m*M* dendrimer-C_{12} in 20 m*M* borate, pH 10.2, 400 V/cm in 20% methanol, pH 10.8, 500 V/cm in 40% methanol, pH 11.0, 500 V/cm in 60% methanol, pH 11.2, 500 V/cm in 80% methanol. Reprinted with permission from J. Chromatogr. [57].

concerns with the analysis of "dirty" samples or samples containing strongly retained compounds, which could contaminate the packed capillaries employed in CEC.

However, mass spectrometric detection is still more complicated with electrokinetic chromatography due to the presence of the ionic polymers in the buffer medium and due to the potential interference of low-molecular-mass impurities in the polymeric pseudo-stationary phase. Additionally, gradient elution is a viable option in CEC [99], but may be more difficult to implement in electrokinetic chromatography due to the limited migration range.

7. Summary

The results to date have demonstrated the utility of micelle polymers and their advantages over conventional micelles as pseudo-stationary phases for MEKC. In spite of early concerns about mass transfer and polydispersity and their effects on

efficiency, separations using the polymers do not necessarily suffer from reduced plate counts. Overall, the chromatographic performance and chemical selectivity of the polymeric pseudo-stationary phases is very good, with the polymers often providing unique selectivity and broad migration range. The advantage of greater stability has provided the capability of separating hydrophobic compounds in the presence of high concentrations of organic modifiers. The lack of a critical micelle concentration of free surfactant has improved chiral separations, and has permitted the introduction of mass spectrometric detection. Still, research has only begun into the application of micelle polymers, surfactant polymers, and dendrimers as pseudo-stationary phases.

8. Future directions

The future of this area of electrokinetic chromatography is exciting. The studies to date have employed a relatively small number of polymer structures for a limited number of separations. Further work in this area should concentrate on the introduction and characterization of new polymeric pseudo-stationary phases, new applications of micelle polymers, fundamental characterization of the interactions between polymers and analytes and the effect of polymer structure and solvation on these interactions, refinement of the use of mass spectrometric detection for MEKC, and application of micelle polymers in areas where packed capillaries (CEC) are difficult to prepare or maintain.

The studies to date have only begun to explore the myriad of possibilities of micelle polymers and polysoaps that are available. Many polymer micelles and polysoaps have been reported in the literature for a variety of commercial applications [73,74], and these structures might be adapted to MEKC to provide a wide range of chemical selectivities. It should be possible to synthesize and employ polymers with unique selectivity, high electrophoretic mobility and monodisperse structures. An obvious extension of the work with poly(sodium undecenyl-L-valinate) is the synthesis and polymerization of the undecenoxycarbonylvaline derivative. Conventional micelles of dodecyloxycarbonylvaline have been shown to provide better chiral selectivity than micelles of dodecanoylvaline [23]. As a long-term effort, it may be possible to employ molecularly imprinted polymers for extremely selective separations.

Fundamental studies should concentrate on understanding of the effects of polymer structure on performance. This will facilitate the development and introduction of new polymeric pseudo-stationary phases. An obvious study in this area would be to investigate the chemical interactions between dendrimers and solutes in greater detail, as these phases appear to provide unique selectivity. Studies of this type will provide information regarding the chemical interactions between solutes and polymers, which may be useful for a variety of commercial applications.

An area of application which seems ideal for micelle polymers is environmental analysis [100]. The polymers provide the capability of separating hydrophobic compounds, and the ability to use mass spectrometric detection. Both of these are desirable for many environmental analyses.

Finally, an area where polymeric pseudo-stationary phases appear to have significant advantages over packed capillaries is in the development of miniaturized instruments with micro-machined channels. CE can be carried out on a single chip using this technology, but packing the micro-machined channels with particles is difficult at best with current technology. If polymeric pseudo-stationary phases were to be employed, separations of non-ionic compounds and separations with unique selectivity could be achieved using these miniaturized instruments.

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