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Review

Selectivity of polymeric and polymer-supported pseudo-stationary phases in micellar electrokinetic chromatography

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Abstract

Micelle polymers, polymer surfactants, and dendrimers have recently been employed as pseudo-stationary phases in electrokinetic chromatography. Several advantages over conventional surfactant micelles have been demonstrated. These phases are effective for the separation and analysis of hydrophobic compounds and chiral compounds, and the application of mass spectrometric detection. Additionally, the polymeric phases often demonstrate unique selectivity relative to micellar phases, and can be designed and synthesized to provide desired selectivity. This review covers efforts to characterize the selectivity of polymeric pseudo-stationary phases since their introduction in 1992. Some thoughts on future development of polymeric pseudo-stationary phases with unique selectivity are presented. © 1997 Elsevier Science B.V.

Keywords: Selectivity; Pseudo-stationary phases; Micelles; Reviews; Surfactants; Dendrimers; Alkyl phenyl ketones; Polynuclear aromatic hydrocarbons; Benzenes

Contents

1. Introduction

Introduced in 1984 by Terabe et al. [1], micellar electrokinetic chromatography (MEKC) is a modification of capillary electrophoresis 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 such as sodium dodecyl sulfate (SDS) migrate at a rate slower than that of the electroosmotic flow. The rate

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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]).

Conventional micelles are very useful as pseudo-stationary phases. By far the most common surfactant employed is SDS, which has a low critical micelle concentration (CMC) and which provides good selectivity and efficiency. Many other commercial surfactants with varying selectivity are available to be employed as pseudo-stationary phases [6–8].

However, significant limitations result from the use of conventional micelles as pseudo-stationary phases in MEKC. Conventional micellar pseudo-stationary phases have limited stability, being in a state of equilibrium with the free surfactant in the surrounding buffer medium. This limits the flexibility of MEKC in terms of the choice of analytical conditions. For example, hydrophobic analytes are difficult to separate because they tend to have migration times close to t_{mc} with very high retention factors, but adjustment of the retention factors by the addition of organic solvents disrupts micelle formation. The use of conventional surfactants limits the applicability of MEKC for mass spectrometric detection unless the surfactant is somehow removed, because the presence of a high concentration of low-molecular-mass surfactant leads to large background signals in the low-molecular-mass region of the mass spectra. Finally, commercial surfactants have not been developed with chromatographic selectivity in mind, and introducing unique or desired selectivity requires either the use of additives or the synthesis of selective surfactants. These limitations have led many researchers to seek alternative pseudo-stationary phases.

The use of polymeric pseudo-stationary phases addresses many of the problems associated with conventional micelles [9]: polymers provide very stable pseudo-stationary phases with zero CMC; with zero CMC, the phases can be used at virtually any concentration; the primary covalent 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 concentrations of organic modifier [10–21]; and they can be employed with mass spectrometric detection [22]. 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 properties desirable in a pseudo-stationary phase. 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 could lead to diminished plate counts relative to conventional micelles, although polydispersity appears to have a minimal effect [20,43].

Several types of alternative pseudo-stationary phases have been employed. Neutral phases have been employed to provide selectivity for ionic compounds. Cyclodextrin polymers [23–25] have been used to provide chiral selectivity, and polyvinylpyrrolidone [26–31] to separate diastereomeric derivatives of enantiomers. Proteins [32–35] and charged cyclodextrins [36] have also been employed for chiral separations. Resorcarenes [37] are stable structures that permit the separation of hydrophobic compounds, but which are limited by background UV absorbance. Dendrimers [10,38–41] and modified dendrimers [21,42] have been utilized as monomolecular pseudo-stationary phases, often with unique selectivity. Covalently stabilized high-molecular-mass surfactants, or micelle polymers, have also been employed as pseudo-stationary phases in MEKC [8,11–20,22,43–49]. These are amphophilic polymers with both hydrophilic and hydrophobic regions. 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. Micelle polymers are thought to maintain micellar form in aqueous media, but can be solvated in organic modified media to take on a structure similar to other high-molecular-mass surfactants. A recent review covers the introduction and development of micelle polymers, high-molecular-mass surfactants and dendrimers in greater detail [9]. This review concentrates on the selectivity of polymeric phases and addresses the origin of differences in selectivity between polymers and micelles.

In the development and characterization of polymeric pseudo-stationary phases, it has often been noted that these phases afford unique selectivity

relative to micelles of SDS [8,11,12,16–21] or of the monomer [11]. The fundamental difference between polymers and conventional micelles is the presence of covalent bonds between the hydrophobic unit structures in the polymer, whereas in micelles the monomers self associate through hydrophobic interactions. The covalent bonds provide the polymer with a fixed primary structure: variation in the size and structure of the polymers is limited to the molecular size of the polymer. Covalent stabilization results in a more structured phase with greater steric constraints than micellar phases. This greater structural rigidity may lead to unique structural selectivity. However, this rigidity may also diminish the ability of the polymer to create suitable hydrophobic domains for the solvation of some hydrophobic compounds, and may limit certain interactions through steric hindrance.

Relative to conventional micelles, there are more variables which may affect the selectivity of separations performed with polymeric pseudo-stationary phases. Because the requirement of self-association is eliminated, micelle polymers with varied structure and chain length can be employed. Polymers with alkyl chains as short as four carbons have been employed. The alkyl chain length and ionic head group chemistry can be varied while keeping the backbone structure of the polymer constant. This is comparable with liquid chromatography, where a given support material can be modified with a variety of agents to provide stationary phases with dramatically different selectivity. Additionally, due to the stability of the polymeric phases, the effects of organic modifiers in the run buffer can be studied without altering the primary covalent pseudo-stationary phase structure.

Two recent reviews have covered the origins, structure and properties of micelle polymers and polymer surfactants in great detail [50,51]. Although these polymers are often developed as solubilizing agents, limited studies have been performed on the chemical interactions between the polymers and solubilizates. It may be seen as an advantage of MEKC that the chemical interactions between polymers (or micelles for that matter) and solubilizates can be probed.

This review concentrates on the chromatographic selectivity of polymer surfactants, micelle polymers and dendrimers when employed as pseudo-stationary phases in MEKC. 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-undecenoylvaline) and acrylate polymers.

In general, limited studies of the selectivity of polymeric pseudo-stationary phases have been performed. Only one study has used linear solvation energy relationships (LSER) to characterize a high-molecular-mass surfactant [8]. Other studies have relied on more general selectivity measures.

It is important in selectivity studies in MEKC to interpret the results in terms of the selectivity between analytes in a given separation. The retention factor,

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

(where t_{mig} is the migration time of the analyte, t_0 is the migration time of a compound which does not interact with the pseudo-stationary phase, and t_{mic} is the migration time of the micelle) is a function of the affinity of the pseudo-stationary phase for the analyte and the phase ratio. Because it is difficult or impossible to match the phase ratio when comparing two pseudo-stationary phases, the selectivity,

$$\alpha = \frac{k_2}{k_1}$$

between analytes within a separation must be compared.

The methylene selectivity, α_{CH_2}, is often reported. This is the selectivity between adjacent pairs in an homologous series: the selectivity between two compounds which differ only by the presence of a methylene group. This is generally accepted as a measure of the hydrophobicity of the pseudo-stationary phase, with greater methylene selectivity indicating greater hydrophobicity. In many studies the logarithm of the retention factor for a series of compounds on one pseudo-stationary phase is plotted vs. the logarithm of the retention factor on a second pseudo-stationary phase. When the selectivity of the phases is the same, the points are expected to fall on

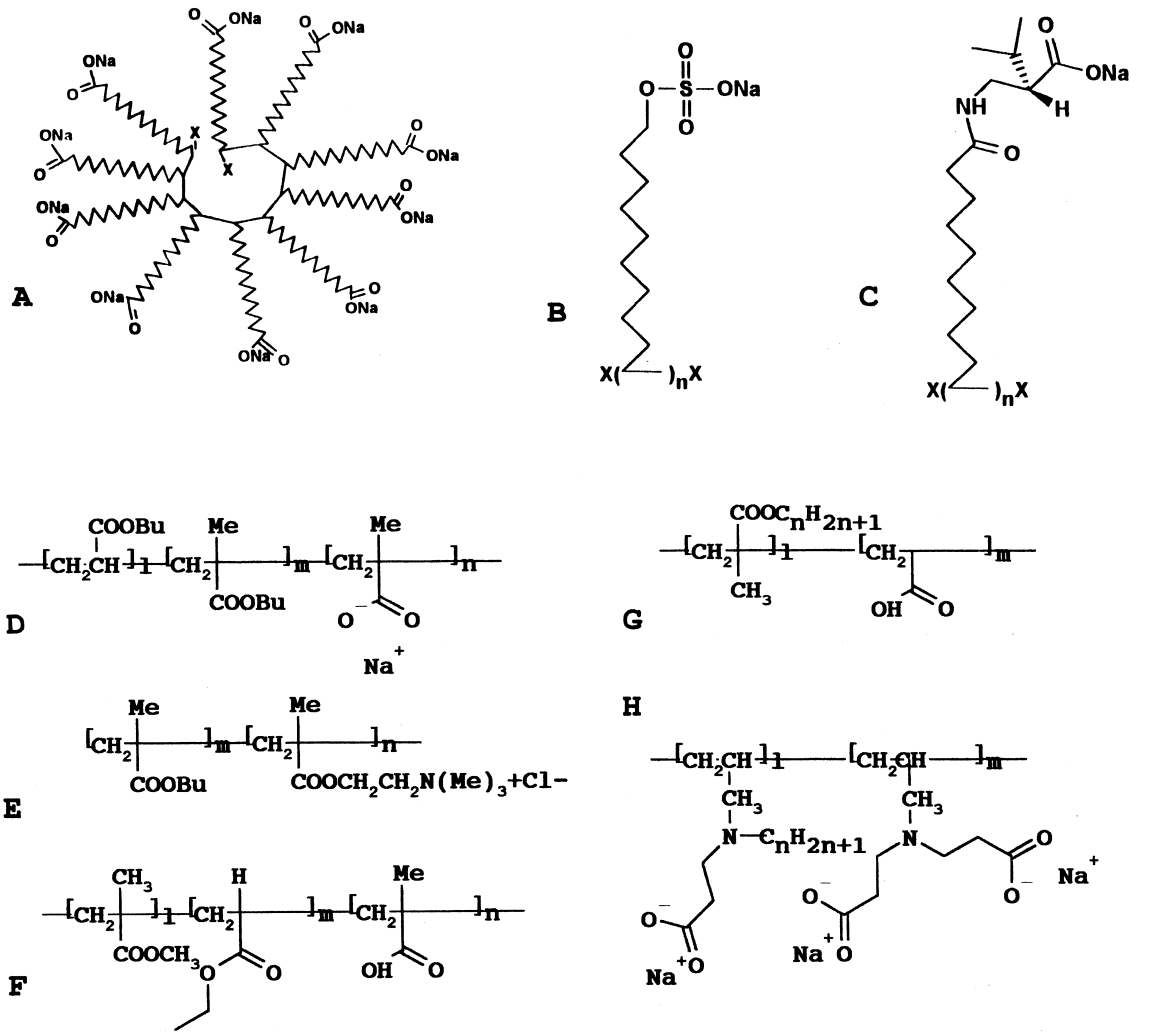

Fig. 1. Structures of the pseudo-stationary phases. (A) Poly(sodium undecylate), (B) poly(sodium undecenyl sulfate), (C) poly(sodium undecenoylvaline), (D) BBMA, (E) BMAC, (F) Elvacite 2669, (G) alkylmethacrylate methacrylic acid copolymers, (H) alkyl substituted polyallylamines. Structures of the dendrimers are presented in Fig. 11.

a straight line. If the selectivity is different, a scatter plot is expected. Differences in log k are directly related to selectivity:

$$\log k_2 - \log k_1 = \log \frac{k_2}{k_1} = \log \alpha$$

and thus these plots are good indicators of overall selectivity. Further information can be deduced from the plots by observing the relative position of various classes of analytes.

2. Poly(sodium 10-undecylenate) and poly(sodium 10-undecenylsulfate)

The first successful reports of the use of a micelle polymer were those of Palmer et al. [11,12]. These authors used a micelle polymer of sodium-10-undecylenate (SUA, Fig. 1A) as a pseudo-stationary phase to achieve the separation of alkyl phthalates and polynuclear aromatic hydrocarbons (PAHs) in buffers modified with up to 50% methanol or 45% acetonitrile.

The polymer was observed to have unique selectivity relative to micelles of SDS or of the monomer [11,12], as determined by the relative migration times. The polymer was more retentive of polar compounds, and less retentive of non-polar compounds. At this point it was unclear what was causing this difference in selectivity. Steric constraints of the polymer might prevent formation of a hydrophobic domain capable of solvating the hydrophobic compounds studied. Alternatively, the differences observed may have been caused by the presence of polymer end groups (either sulfates or hydroxyls) on the interior of the polymer, or the difference in head group chemistry between the polymer and SDS micelles.

The electrophoretic mobility of the SUA polymer increases substantially between 30% and 40% acetonitrile and plots of log k vs. percent acetonitrile are non-linear [12]. The k values of all of the analytes studied were affected to nearly the same extent, resulting in no dramatic change in the selectivity of the polymer. The increase in mobility and apparent change in interaction with analytes were interpreted to mean that the structure and solvation of the polymer are dynamic. The change in the structure of the polymer results in greater mobility at high acetonitrile concentrations either through reducing the Stoke's radius of the polymer or by exposing more ionic head groups. Additionally, the change in structure of the polymer changes the nature of the interaction between the analytes and the polymer, resulting in non-linear plots of log k vs. percent acetonitrile. A reasonable hypothesis is that in the absence of organic modifier the polymer maintains a collapsed or entangled structure which minimizes interactions between water and the hydrophobic alkyl chains, while in the presence of acetonitrile the polymer can assume a more open structure with the alkyl chains solvated by the acetonitrile.

Partly to eliminate the problems with the carboxylate head group, and partly to prepare a micelle polymer with the same head group chemistry as SDS, Palmer and Terabe synthesized and employed poly(sodium undecenyl sulfate) (SUS, Fig. 1B), the sulfate analog of poly(sodium undecylenate) [13,16,17]. Shamsi et al. also utilized this polymer for the separation of PAHs and with cyclodextrins for chiral separations [52]. Like its carboxylate counterpart, this polymer provided efficient and selective separations of a variety of compounds in aqueous and modified aqueous buffers [16,17,38]. The chemical selectivity of the polymer was studied using a series of substituted benzene and naphthalene compounds, and the structural selectivity was studied using PAHs [17].

A comparison of the separation of substituted benzene and naphthalene compounds achieved with the sulfate polymer and SDS micelles is shown in Fig. 2. Changes in migration order indicate the different selectivity of the polymer and the SDS micelles. Plots of log k on the polymer phases vs. log k using SDS micelles, shown in Fig. 3, indicate that both polymers interact more strongly with compounds having amine or hydroxyl groups, implying that they are more polar or are better hydrogen bond acceptors or donors. A plot of log k on the undecylenate phase vs. log k on the undecenyl sulfate phase, shown in Fig. 4, shows that the selectivity of the two polymers is virtually identical. This clearly illustrates that the differences in selectivity between the polymers and SDS micelles are not due to the structure of the ionic head group.

In further work carried out in Palmer's laboratory [53], three different initiators were employed to initiate polymerization of sodium undecylenate. All previous studies had employed ammonium persulfate [11–13,16,17] or UV irradiation [52] to initiate polymerization. Different initiators were studied in part to determine the effect of initiator structure on the selectivity of the polymer phases. Fig. 5 shows the structures of the initiators, and Table 1 summarizes the results. The polymer initiated with 2,2′-azobis(2,4-dimethylvaleronitrile) was not soluble in aqueous buffers. The methylene selectivity is lower on the phase initiated with 2,2′-azobis(2-methylpropionitrile) (AIBN), a more hydrophobic initiator, a result which was not expected. The polymers initiated with hydrophobic initiators also had lower molecular masses, probably due to faster termination by the hydrophobic initiators. Fig. 6 is a plot of log k using the AIBN initiated polymer vs. log k using the ammonium persulfate initiated polymer. The overall selectivity is not significantly different ($r^2 = 0.998$, $m = 0.962$). These results prove that the end groups on the interior of the polymer are not a significant factor causing the stronger interac-

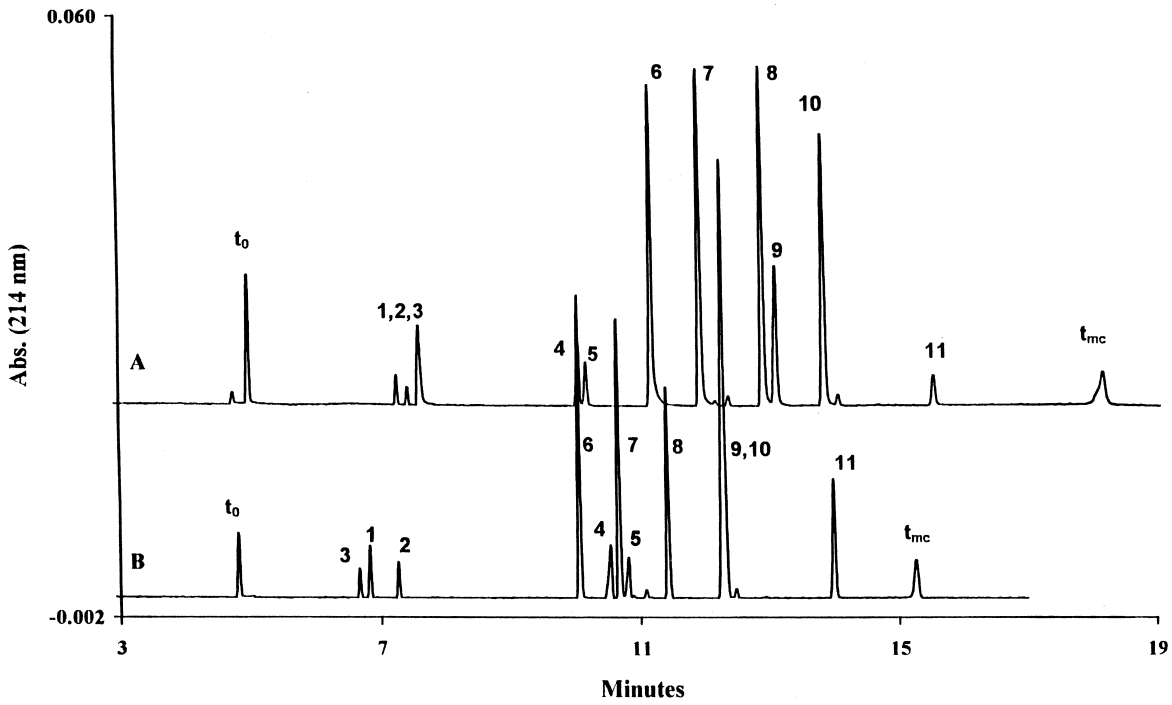

Fig. 2. Separation of substituted benzene and naphthalene compounds. (A) 0.83% SUS polymer, (B) 30 m*M* SDS; capillary is 50 cm effective length and 57 cm total length, 16.1 kV applied potential. A phosphate–borate buffer at pH 7.3 was employed; 1 = nitrobenzene, 2 = anisole, 3 = *p*-nitroaniline, 4 = *o*-xylene, 5 = *m*-xylene, 6 = naphthylamine, 7 = naphthalenemethanol, 8 = acenaphthenol, 9 = naphthalene, 10 = naphthaleneethanol, 11 = diphenyl ether. Reprinted with permission from [17], copyright 1997, American Chemical Society.

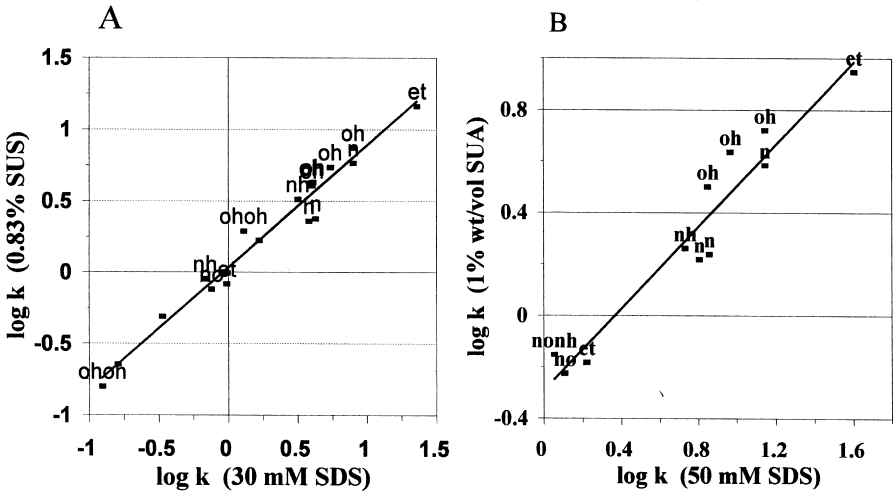

Fig. 3. Logarithm of the retention factors for the analytes in Fig. 2 and selected cold medicine ingredients using (A) 0.83% SUS polymer vs. 30 m*M* SDS in pH 7 phosphate–borate buffer (reprinted with permission from [17], copyright 1997, American Chemical Society) and (B) 1% (w/v) SUA vs. 50 m*M* SDS in pH 8.4 phosphate–borate buffer. Labels are as follows: et = ether group, n = functioal groups, nh = amine group, oh = hydroxyl, ohoh = two hydroxyl groups. Unlabeled points have multiple functional groups. Cold medicine ingredients and some alcohols were not studied with SUA because they are ionic at pH 8.4.

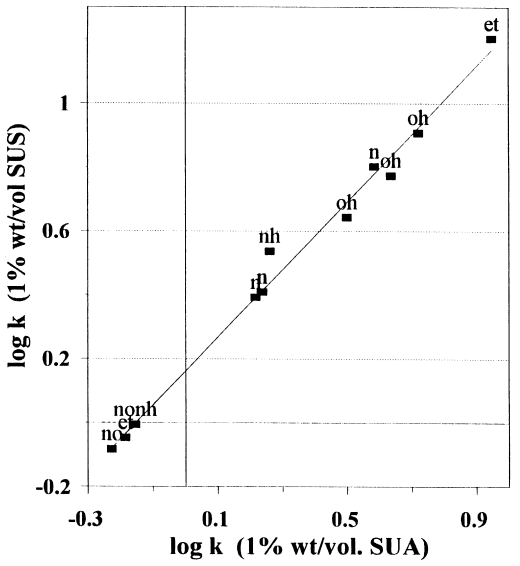

Fig. 4. Logarithm of the retention factors for the analytes in Fig. 2 using 0.83% SUS polymer vs. 1% (w/v) SUA in pH 8.4 phosphate–borate buffer; labels are as in Fig. 3 (reprinted with permission from [17], copyright 1997, American Chemical Society).

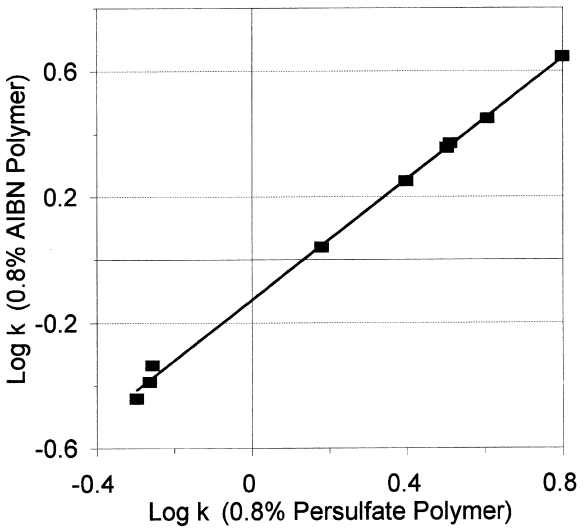

Fig. 6. Logarithm of the retention factors for the analytes in Fig. 2 (without the xylenes) using 0.8% AIBN initiated SUA polymer vs. 0.8% ammonium persulfate initiated SUA polymer in borate buffer (pH 9).

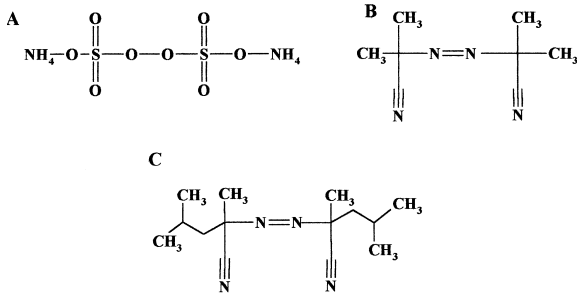

Fig. 5. Structures of three free radical polymerization initiators used to initiate polymerization of sodium undecylenate: (A) ammonium persulfate, (B) AIBN, (C) 2,2′-azobis(2,4-dimethylvaleronitrile).

tions with polar analytes. This fact implies that interaction with the end-group functionality in the rigid core of the polymer micelles is sterically restricted.

The greater interaction between these polymers and polar compounds, and the lower methylene selectivity of these polymers relative to SDS micelles, are indications of lower overall hydrophobicity of the polymer. This may be the result of the smaller size and shorter alkyl chain length of the SUA and SUS polymers relative to SDS micelles. The fact that polymers initiated with hydrophobic initiators had both lower molecular masses and lower methylene selectivity is evidence that the size of the polymer may play a role in determining the strength of the hydrophobic interactions. The more rigid

Table 1
Methylene selectivity results for SUA polymers initiated with AIBN and ammonium persulfate

	AIBN initiated SUA polymer	Ammonium persulfate initiated SUA polymer
α_{CH_2} Alkyl phenyl ketones	1.92±0.02	1.99±0.02
α_{CH_2} Alkyl benzoates	1.964±0.005	2.016±0.005

covalent structure of the polymer may also hinder suitable interactions with flexible hydrophobic compounds.

The SUS polymer is also useful in buffers modified with organic solvents, and the selectivity of the polymer is rather interesting. Relative to micelles of SDS, the SUS polymer retains greater methylene selectivity in buffers modified with acetonitrile and methanol [17]. The structural selectivity has also been studied [17]. 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 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. Acetonitrile is a stronger solvent, and may lead to a larger hydrophobic region due to better solvation. As a stronger solvent, acetonitrile may also lead to a more structured conformation in the alkyl chains or polymer backbone.

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 [54–62]. Wang and Warner [44,47], Dobashi et al. [45], Agnew-Heard et al. [47] and Williams et al. [48] have reported modification of sodium undecylenate with L-valine to obtain a chiral surfactant. The surfactant was then polymerized by gamma [44,46–48] or UV [45] irradiation to obtain the chiral micelle polymer (Fig. 1C). This is the first instance of a polymeric pseudo-stationary phase with chiral selectivity.

Poly(sodium undecenoyl-L-valine) and a cationic amide of this compound have been synthesized, polymerized, and employed for chiral separations. The two structures were patented for use in chiral separations by electrokinetic chromatography in 1992 [63,64].

Wang and Warner investigated further the use of this polymerized chiral micelle in 1994 [44]. 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-D-valine) was employed as a pseudo-stationary phase. The polymer has been employed in combination with γ-cyclodextrin, with the combined effect of the chiral polymer and the chiral recognition of the cyclodextrin provided greatly improved separations (chiral resolution of 2.5 to 6.5) of (±)-1,1′-binaphthol, (±)-verapamil, (±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate and D,L,-laudanosine [46]. The micelle polymer, because of its greater size, cannot be included in the CD cavity and does not interfere with the chiral recognition of the cyclodextrin. Employed without cyclodextrins, the polymer has been shown to provide selectivity for Tröger's base, binaphthyl, paveroline and coumarin derivatives [47]. 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. Elimination of the dynamic equilibrium associated with conventional micelles was the explanation given for better separations obtained with the polymerized micelle. The authors report better separations at pH 10 than at pH 9, which they attribute to a more open structure of the polymer at higher pH [65] which leads to better interactions. 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 poly(vinyl 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.

Dobashi et al. concentrated on the separation of dinitrobenzoyl amino acid isopropyl esters and compared the separations achieved with micelles formed from chiral surfactants to those obtained with the chiral micelle polymer [45]. 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 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.

The work with poly(sodium undecenoylvaline) proves that polymers can be synthesized with the selectivity desired. That the migration order of chiral compounds can be reversed by using the D-valine vs. the L-valine derivative is a powerful demonstration of this fact. Sodium undecenoylvaline was a convenient monomer for this work, but the approach may not be the most effective. No studies have been reported to date with other specially synthesized chiral polymers, and it is unknown what the most appropriate head group or backbone structure may be. This is an area which deserves greater attention.

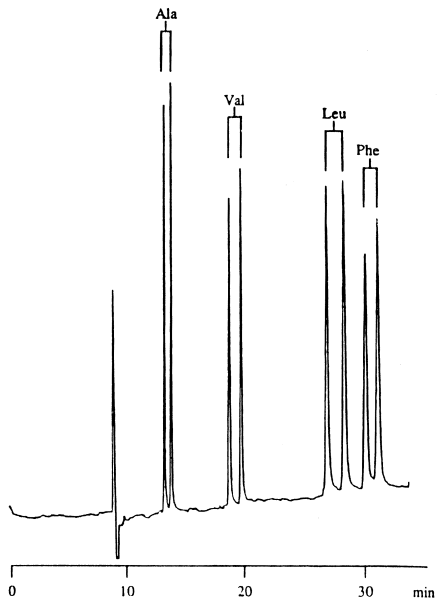

Fig. 7. Separation of a mixture of four enantiomeric (3,5-dinitrobenzoyl)amino acid isopropyl esters with poly(sodium undecenoyl-L-valinate) solution by electrokinetic chromatography. Conditions: 50 cm×50 μm I.D. fused-silica capillary, applied potential 12.4–12.8 kV, UV detection at 254 nm; 0.76 poly(sodium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

4. Acrylate copolymers

Polymer surfactants with the forms shown in Fig. 1D–G have been employed for MEKC separations of cold medicine ingredients, [32,43] substituted benzenes [8,32,43], substituted naphthalenes [32,43] and hydrophobic compounds (PAHs, *n*-alkylphenyl ketones, fullerenes) [15]. The polymers have also been employed with cyclodextrins for the separation of dansyl amino acids [14] and for MEKC with mass spectrometric detection [22]. The chemical selectivity of these polymers has also been studied in some detail [8].

Terabe et al. [32] and Ozaki et al. [43] were the first to report the use of an acrylate copolymer as a pseudo-stationary phase. They employed butyl acrylate–butyl methacrylate–methacrylic acid (1D, BBMA) copolymers for the separation of benzene derivatives, cold medicine ingredients, and naphthalene derivatives. 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 naphthalenemethanol or naphthaleneethanol, which was not observed with SDS micelles.

pH values and polymer structures were also shown to affect the selectivity and performance of the polymer. From pH 4 to 7 the electrophoretic mobility of the polymer increased 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 electrophoretic mobility was greater and the retention factors were lower. It was concluded by the authors that it is more suitable to change the polymer chemistry than to change the pH, since at pH values below 4 the polymer precipitates and 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.

In further work with BBMA, Ozaki et al. [14] investigated the effects of the addition of methanol and a non-ionic surfactant, octaoxyethylene-dodecanol ($(EO)_8R_{12}$). 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 non-ionic surfactant forms comicelles with the polysoap, rather than forming independent non-ionic micelles. If independent micelles had been formed, the retention factors would have been reduced by competitive partitioning into the non-ionic 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 [14]. In combination with 10 m*M* ß-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* ß-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, which can be co-included in the cyclodextrins [65–69], reducing chiral selectivity. The BBMA cannot 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) [14]. The migration order of substituted naphthalene compounds was found to be similar to that with BBMA, but significantly different from that with SDS micelles.

Yang et al. 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 [15], and used linear solvation energy relationships (LSER) to characterize the chemical selectivity of this polymer relative to several conventional micelles [8]. LSER studies using 60 aromatic test solutes were used to measure the relative cohesiveness, hydrogen bond acceptor strength, and hydrogen bond donor strength of Elvacite 2669 [8]. Cohesiveness, or resistance to cavity formation, and hydrogen bond donor strength were found to be the most important contributors to the selectivity of Elvacite 2669, while the hydrogen bond acceptor strength plays a minor but significant role. This is similar to the retention behavior of SDS and sodium cholate micelles. The polymer was found to have cohesiveness between hydrocarbon micelles (less cohesive) and fluorocarbon micelles (more cohesive), although the cohesiveness is more similar to the fluorocarbon micelles. 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. It is interesting that Elvacite 2669 has intermediate properties relative to those of conventional micelles, indicating that sub-

stantially 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 might be expected from the more rigid covalent structure of the polysoap, which would require greater energy to rearrange to solubilize larger analytes.

In additional work [15], the migration behavior of the 60 test compounds was studied in detail on Elvacite 2669 relative to SDS micelles by plotting log *k* with the polymer vs. log *k* with 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. In many cases, the compounds which interact more strongly with Elvacite 2669 are the same compounds which interact strongly with SUA and SUS polymer micelles.

Palmer has recently studied a series of acrylate copolymers with differing alkyl chain lengths and molecular masses [18]. The polymers are of the structure shown in Fig. 1G, with alkyl chain lengths of nine (C_9), thirteen (C_{13}) and eighteen (C_{18}) carbons. All of the polymers had the same acrylate/alkyl acrylate mole ratio and approximately the same molecular mass, and thus changes in selectivity were solely due to the differences in alkyl chain length. The C_{13} polymer was studied with two molecular masses. The results of this study are summarized in Table 2. As expected, the methylene selectivity increased with increased alkyl chain length, because the polymers become more hydrophobic. Relative to SDS micelles, the polymers progressed from having greater overall interaction with polar compounds (C_9) to having greater overall interaction with nonpolar or hydrophobic compounds (C_{18}). The exception to this rule was the amine compounds, which invariably interact more strongly with the polymer phases. The amines are hydrogen bond acceptors, and the strong interaction indicates that the polymers are in general stronger hydrogen bond donors than SDS micelles. The C_{13} polymer had selectivity most similar to SDS micelles. Fig. 8 shows a plot of log *k* on the C_9 phase vs. that on the C_{18} phase, demonstrating that significant differences in selectivity are realized by varying the alkyl chain length. In several instances (*p*-nitroaniline, nitrobenzene, anisole and *o*-xylene), the migration order of analytes was reversed as the alkyl chain length progressed from nine to eighteen carbons or between the acrylate polymers and SDS micelles (naphthylamine and naphthalene-methanol).

No significant differences in selectivity were observed between the two C_{13} polymers with different molecular mass. Higher molecular mass did, however, reduce the solubility of the polymer and increase the viscosity of the polymer solution.

Plots of log *k* vs. carbon number for homologous series of alkylphenyl ketones and alkyl benzoates in aqueous buffers, which are generally linear [70], were not linear for the C_{13} and C_9 polymers. Negative deviations were observed for homologues with longer alkyl chains (four to six carbons), and the deviations were more severe with the C_9 phase

Table 2
Methylene selectivity results for acrylate copolymers Fig. 1G with different alkyl chain lengths

	C_9	C_{13}	C_{13} high M_r	C_{18}
α_{CH_2} Alkyl phenyl ketones	1.49	2.18	2.11	2.57
α_{CH_2} Alkyl benzoates	1.52	2.18	2.10	2.62

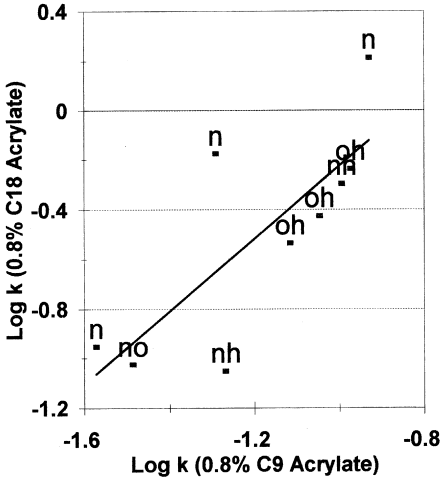

Fig. 8. Logarithm of the retention factors for the analysis in Fig. 2 (without the xylenes) using 0.8% C_{18} acrylate copolymer vs. 0.8% C_9 acrylate copolymer in borate buffer (pH 9).

than with the C_{13} phase. The C_{18} phase did yield linear plots. Evidently, homologues with longer alkyl chains are not well solvated by polymers with shorter alkyl chains, implying limited ability of these polymers to create a large hydrophobic domain.

The study by Palmer is also evidence that a given polymer backbone can be modified to provide unique selectivity. In principle, polymeric pseudo-stationary phases can be designed and synthesized to provide the selectivity desired for a particular separation.

5. Polyallylamine supported phases

Tanaka et al. have recently studied polyallylamine (PAA) supported pseudo-stationary phases (Fig. 1H) [19,20]. These polymers were synthesized with varying alkyl chain lengths, and different degrees of alkylation. The polymers were studied in methanol-modified buffers for the separation of alkyl phenyl ketones and PAHs.

Limited selectivity studies have been performed with these polymers. The methylene selectivity results are presented in Table 3. The methylene selectivity of PAA modified with dodecyl chains is similar to that of SDS micelles in both aqueous media and 60% methanol. However, the methylene selectivity of PAA modified with hexadecyl chains is higher than that observed with SDS in both 20% and 60% methanol. The methylene selectivity of the hexadecyl-modified polymer is similar to that observed by Palmer with acrylate copolymers with octadecyl chains [18]. Plots of log k vs. carbon number for the alkyl phenyl ketones are not always linear, especially at intermediate methanol concentrations. Again, the non-linearity at low carbon numbers may be explained by the differences in the hydrophobicity of methylene groups near the functional group [70], but deviations for higher carbon numbers must be due to the inability of the polymer to create a large hydrophobic domain capable of solvating long hydrocarbon chains. This is especially true at intermediate concentrations of methanol, where only part of the alkylated polymer is solvated by methanol.

As presented in Fig. 9, the selectivity of hexadecyl-modified PAA is very similar to that for dodecyl-modified PAA for the separation of PAHs and alkyl phenyl ketones in 40% methanol. However, in 60% methanol the selectivity is much different, with the hexadecyl phase showing strong preference for the PAHs. This appears to be due to a change in the selectivity of the hexadecyl phase, which shows strong preference for the PAHs in 60% methanol, but not in 40% methanol (Fig. 10).

As was observed with acetonitrile and the SUA polymer, the electrophoretic mobility of these polymers was observed to increase dramatically at a particular concentration of methanol as a modifier. This increase in electrophoretic mobility provided a wide migration range, and meant that separations of hydrophobic compounds could be optimized in a similar manner to reversed-phase liquid chromatography. Interestingly, the increase in mobility occurred at higher concentrations of methanol for the polymers modified with longer alkyl chains. Additionally, as was observed with the SUA polymer, plots of log k vs. percent methanol were non-linear, indicating a change in the retention mechanism. This was interpreted as a change in the polymer's con-

Table 3
Methylene selectivity for alkyl phenyl ketones of alkylated PAA, alkylated dendrimers and SDS in aqueous buffer and 60% methanol

	α_{CH_2} aqueous buffer	α_{CH_2} 60% methanol
SDS micelle	2.29	1.27
PAA-C_{12} [20]	2.45	1.32
PAA-C_{16} [10]	2.70[a]	1.67
SBD (G=2.5-C_8) G=3.5	1.55	–
SBD (G=3.5-C_8) G=3.5	1.99	–
SBD (G=3.5-C_8) G=4.5	1.72	1.19
SBD (G=2.5-C_{12}) G=3.5	2.13	1.13
SBD (G=3.5-C_{12}) G=3.5	2.32	1.57

[a] 20% methanol.

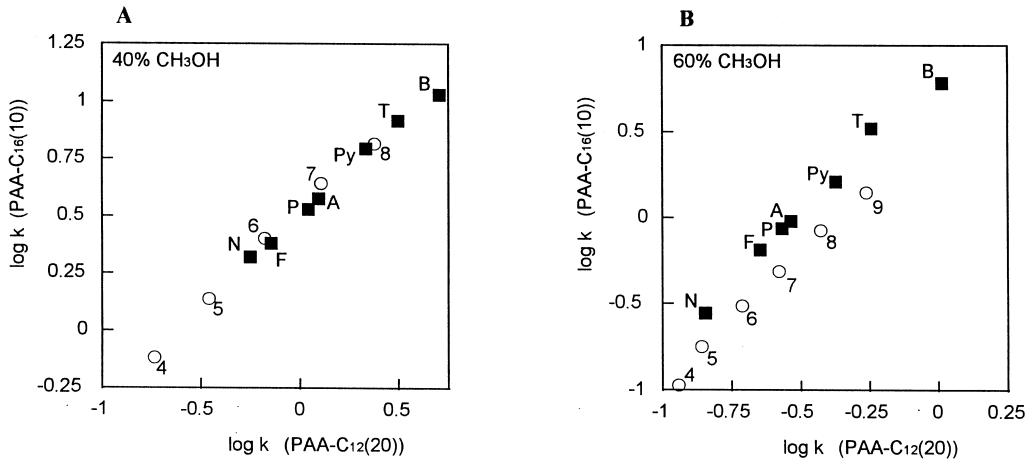

Fig. 9. Logarithm of the retention factors for alkyl phenyl ketones and PAHs using PAA-C_{16} vs. PAA-C_{12} in (A) 40% methanol and (B) 60% methanol. 4–9 refers to the number of carbons in the alkyl phenyl ketone chain and N = naphthalene, F = fluorene, P = phenanthrene, A = anthracene, Py = pyrene, T = triphenylene and B = benzo[*a*]pyrene.

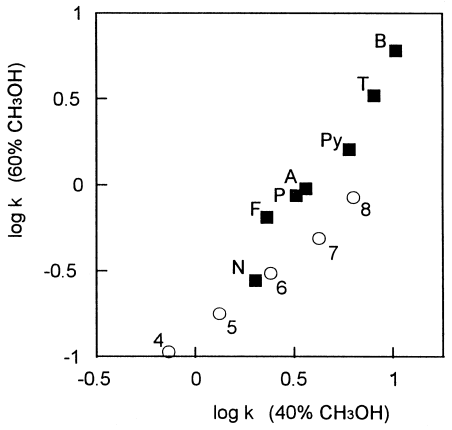

Fig. 10. Logarithm of the retention factors for alkyl phenyl ketones and PAHs using PAA-C_{16} in 60% methanol vs. PAA-C_{16} in 40% methanol; labels as in Fig. 9.

formation or secondary structure with solvation by methanol. Solvation by methanol is said to begin at the less hydrophobic regions, leaving the more hydrophobic regions unsolvated.

6. 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’ [71,72]. Unlike micelles, however, dendrimers become more sterically hindered toward the exterior of the molecule, and the interior may be hydrophobic or hydrophilic. Dendritic molecules have been shown to provide rather unique selectivity. The selectivity is often affected by the presence of internal functionality, such as amine groups. It has also been shown that the selectivity can be altered through modification of the exterior of the dendrimer with alkyl chains of varying length. Fig. 11 shows the general structure of some of the dendrimers used to date as pseudo-stationary phases, as well as the structure of modified dendrimers.

Tanaka et al. were the first to report the use of dendrimers as carriers in electrokinetic chromatography [38]. The selectivity of small polyamidoamine dendrimers was shown to be significantly different from that of SDS or cetyltrimethylammonium chloride micelles.

Kuzdzal et al. employed amide-based cascade

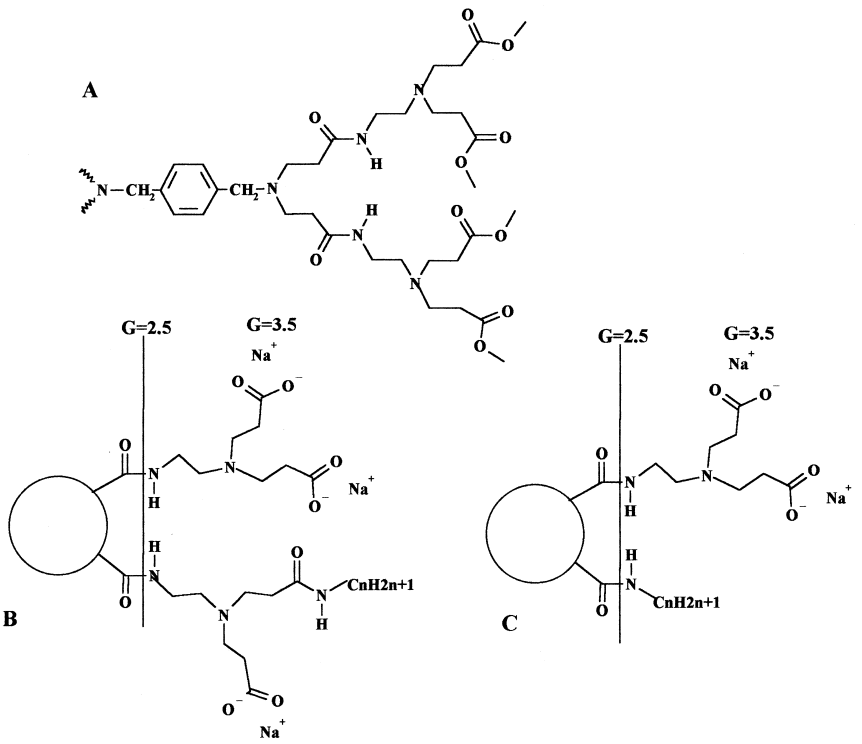

Fig. 11. Structures and notations for the alkyl-modified dendrimers. (A) General structure of the dendrimers with xylenediamine core, (B) SBD (G=3.5-C_n) G=3.5, (C) SBD (G=2.5-C_n) G=3.5. G is the generation of the dendrimer.

macromolecules with carboxylic acid terminus for the separation of alkyl parabens and Robitussen cold medicine ingredients with good selectivity and efficiency [39]. 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 further the structural selectivity of poly(amidoamines) with ammonia and *p*-xylenediamine as starting materials for the separation of alkylphenyl ketones, substituted benzene and naphthalene compounds and aromatic hydrocarbons [10,21]. The selectivity was found to be significantly different from that of conventional micelles. The dendrimers were found to separate benzene derivatives from naphthalene derivatives, but did not separate well the compounds with a difference in functional groups. The contrast in selectivity relative to SDS-MEKC is demonstrated in Fig. 12. Using SDS micelles, benzene derivatives with hydrophobic functional groups migrate after naphthalene compounds with hydrophilic functional groups. With the dendrimer, however, selectivity is between the benzene and naphthalene derivatives. Surprisingly, as demonstrated in Fig. 13, very little selectivity was observed for alkyl benzenes using the dendrimers (methylene selectivity approaches one).

Using half-generation dendrimers, PAHs were separated in 40% methanol and the selectivity was compared to SDS micelles in 60% methanol [10]. The dendrimer preferentially retained the more rigid, compact hydrocarbons relative to SDS micelles. This characteristic of dendrimers can be understood by taking into account the contribution of the hydrophilic, rigid structure of the highly branched polymers [10].

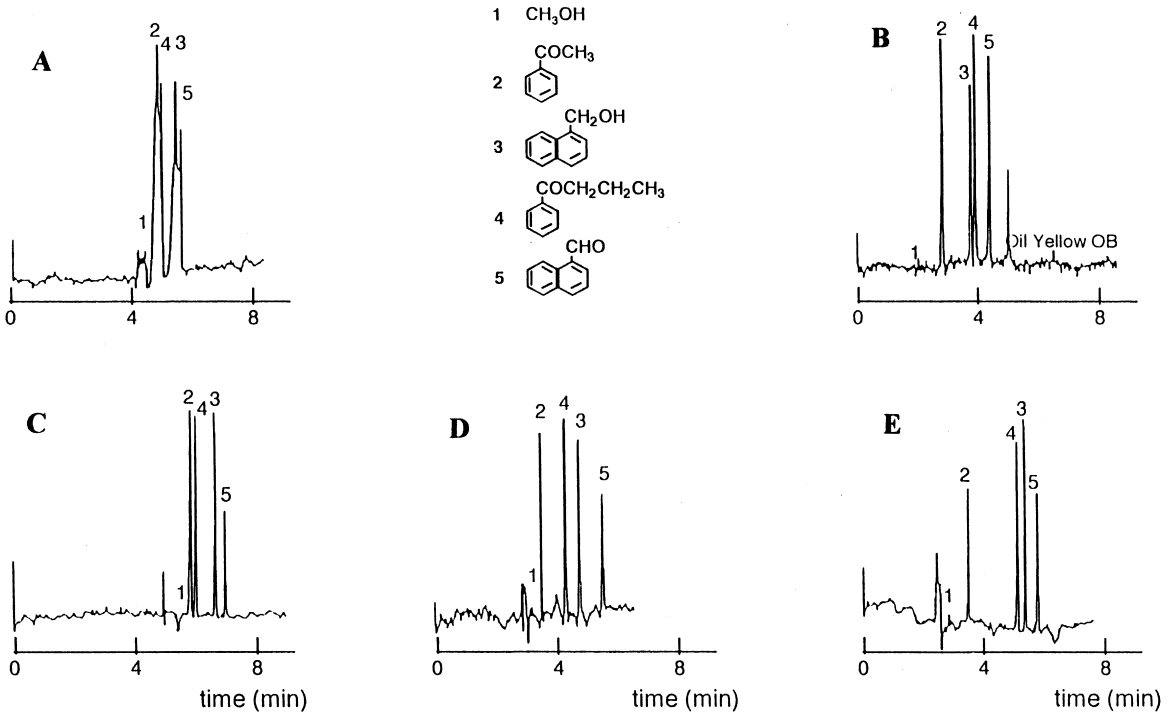

Fig. 12. Comparison of the pseudo-stationary phases for the separation of benzene and naphthalene derivatives. (A) 5 m*M* SBD(X) G=3.5 (pH 10.3), (B) 30 m*M* SDS (pH 9.4), (C) 5 m*M* SBD(X) (G=3.5-C_4 G=3.5 (pH 10.4), (D) 5 m*M* SBD(X) G=3.5-C_8) G=3.5 (pH 10.1) and (E) 5 m*M* SBD(X) (G=3.5-C_{12}) G=3.5 (pH 10.4). Field strength (A to C) 300 V/cm, (D) 250 V/cm. Solutes: (1) methanol, (2) acetophenone, (3) 1-naphthalenemethanol (4) phenyl propyl ketone, (5) 1-naphthaldehyde, (6) oil yellow OB. Buffer solution: 20 m*M* borate, reprinted with permission from [21], Hüthig Publishing Ltd.

Modifying these dendrimers with alkyl chains changes their selectivity, and enhances their utility [21,42]. Half-generation dendrimers can be alkylated by reacting with an alkyl amine, and then hydrolyzed to generate carboxylate end groups. Fig. 11 shows the structure of the alkylated dendrimers. Butyl-, octyl- dodecyl- and tetradecyl-modified dendrimers have been synthesized at different generations. Tetradecyl-modified dendrimers have limited solubility in aqueous and methanol-modified buffers at similar degrees of alkylation as the others.

The degree of alkylation with tetradecyl chains must therefore be very low to be examined as a pseudo-stationary phase. Ethylenediamine and *p*-xylenediamine cores have been employed. Another generation can be added to the dendrimer after alkylation, yielding a polymer with the alkyl groups partially embedded in the core.

All of the modified dendrimers retain greater recognition of backbone structure of analytes, and thus provide unique selectivity relative to SDS micelles. This is demonstrated in Fig. 12, where the alkylated dendrimers show selectivity between that of SDS micelles and the unmodified dendrimers. There is a progression from dendrimer-like selectivity to SDS micelle-like selectivity as the chain length of the alkyl modifiers becomes greater. Alkylated dendrimers provided differentiation between the benzene and naphthalene derivatives than SDS micelles, and greater differentiation between functional groups than the parent dendrimer. Dendrimers modified with dodecyl chains show greater utility than those modified with octyl chains, due to greater recognition of analyte functionality and hydrophobicity.

Separations of alkyl phenyl ketones in aqueous buffers using SDS micelles and alkylated dendrimers are presented in Fig. 13, and the corresponding

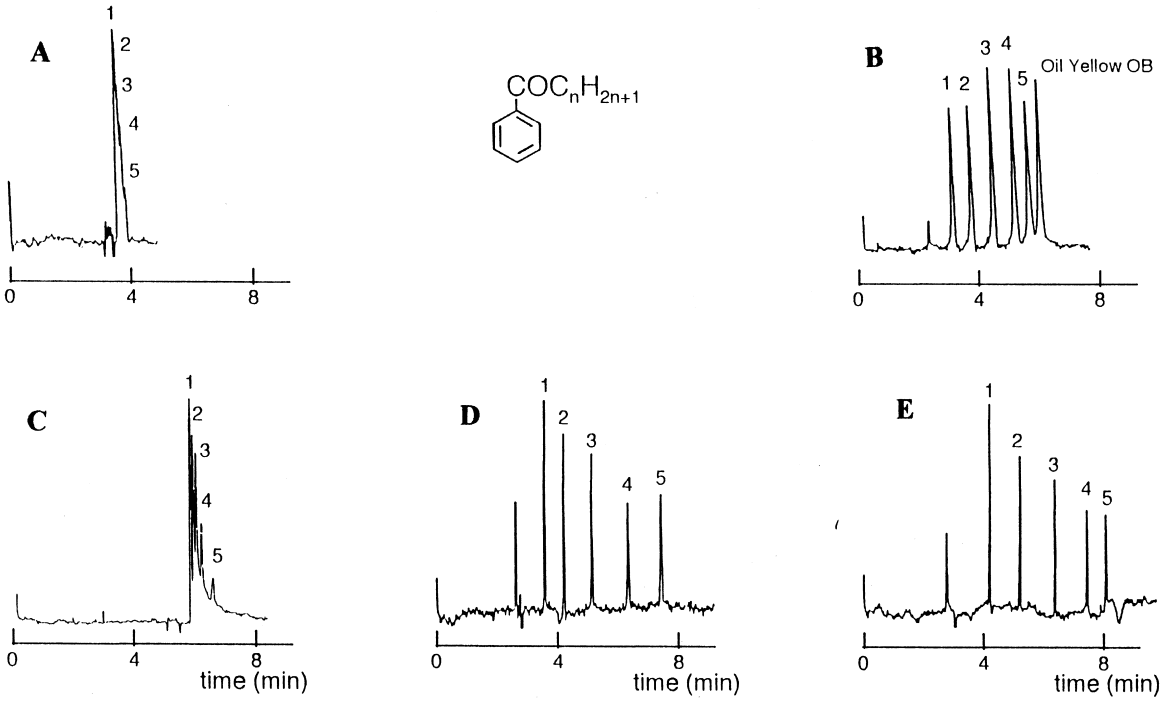

Fig. 13. Comparison of the pseudo-stationary phases for the separation of alkyl phenyl ketones. (A) 5 m*M* SBD(X) G=3.5 (pH 10.3), (B) 30 m*M* SDS (pH 9.4), (C) 5 m*M* SBD(X) (G=3.5-C_4) G=3.5 (pH 10.4), (D) 5 m*M* SBD(X) G=3.5-C_8) G=3.5 (pH 10.1) and (E) 5 m*M* SBD(X) (G=3.5-C_{12}) G=3.5, (pH 10.4). Solute: alkylphenyl ketones. Numbers indicate the numbers of carbon atoms in the alkyl group. Field strength, 300 V/cm. Other conditions as in Fig. 12, reprinted with permission from [21], Hüthig Publishing Ltd.

methylene selectivity results are presented in Table 3. The generation 3.5 dodecyl modified dendrimer shows similar methylene selectivity to SDS micelles in aqueous buffers, but retains greater methylene selectivity in methanol-modified buffers. Octyl-modified dendrimers show less methylene selectivity than SDS micelles in all media, but retain a greater percentage of their methylene selectivity in methanol-modified buffers. Alkylated dendrimers modified with an additional generation after alkylation have lower methylene selectivity than their unmodified counterparts. The methylene selectivity in aqueous media correlates well with the hydrophobicity as measured by the fluorescence of pyrene. The separation of alkylphenyl ketones using SDS micelles shows an abnormal elution profile in 40% methanol, and very narrow migration windows above 40% methanol, while the dodecyl-modified dendrimer shows consistent separation in up to 80% methanol [42]. Plots of log *k* vs. carbon number are generally linear for the alkylated dendrimers.

The structure of the dendrimer core and the generation of the dendrimer have limited effects on the selectivity of the modified dendrimers [21]. No dramatic selectivity differences were observed between the ethylenediamine and *p*-xylenediamine cores. Alkylation at higher generation increases steric crowding of the alkyl chains, and might be expected to affect the selectivity. However, little selectivity differences were observed.

PAHs were also separated using the alkyl derivatized dendrimers. The octyl dendrimer was used in up to 80% methanol, and displayed selectivity similar to the parent dendrimer: planar rigid PAHs were preferentially retained relative to SDS micelles [10]. The dodecyl derivative was employed in up to 90% methanol, and displayed selectivity more like that of SDS micelles [10]. Fig. 14 shows the separation of

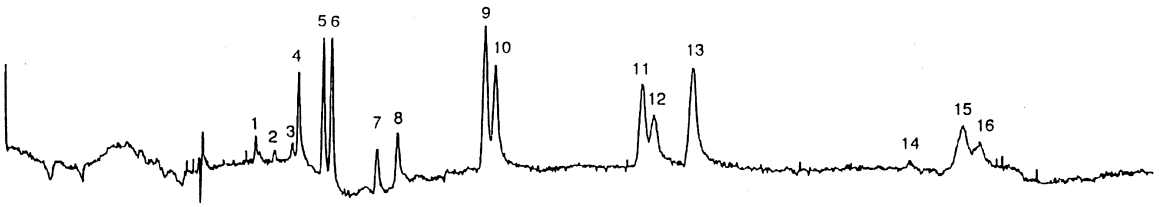

Fig. 14. Separation of PAHs in 65% methanol with 20 m*M* borate buffer. Carrier: 5 m*M* SBD(E) ($G=3.5\text{-}C_{12}$) $G=3.5$ (pH 10.1), 600 V/cm. Solutes: (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) chrysene, (10) benzo[*a*]pyrene, (11) benzo[*b*]fluoranthene, (12) benzo[*k*]fluoranthene, (13) benzo[*a*]pyrene, (14) dibenzo[*a,h*] anthracene, (15) indeno[1,2,3-*cd*] pyrene, (16) benzo[*ghi*]perylene, reprinted with permission from [21], Hüthig Publishing Ltd.

sixteen EPA priority pollutant PAHs with the dodecyl-modified dendrimer in 65% methanol, showing separation of all sixteen analytes.

Plots of log *k* vs. percent methanol on the dodecyl-modified dendrimer were nearly linear, implying a stable structure without conformational or structural change in the presence of high concentrations of methanol [42]. However, an increase in the electrophoretic mobility of the modified dendrimers is observed around 40–60% methanol [21]. This is similar to what was reported with SUA polymer micelles and modified PAA phases, and indicates a conformational or structural change in the polymer backbone as well as in the attached alkyl groups at higher organic modifier concentrations.

Muijselaar et al. have studied the selectivity of a diaminobutane-based poly(propylenimine) dendrimer for the separation of substituted benzene compounds [40,41]. 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 internal tertiary amines, which led to greater interaction with hydrogen bond donating compounds such as hydroquinone and resorcinol.

These results demonstrate the utility of dendrimers as backbone support for the synthesis of pseudo-stationary phases with a wide range of chemical selectivity. 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 may lead to significant improvements in pseudo-stationary phase technology in the coming years. It should be noted, however, that synthesis of the dendrimers is time consuming, and it may be that other polymer backbones will provide an easier route to selective polymeric pseudo-stationary phases [20].

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, especially for the analysis of hydrophobic compounds and chiral compounds. 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 chemical selectivity of the polymeric pseudo-stationary phases is invariably different from that of SDS micelles. It seems likely that the differences in selectivity can often be traced to the more rigid structure of the polymer surfactants, which affects structural selectivity and limits the ability of the polymer to create a large and unstructured hydrophobic domain which is favorable for the solvation of flexible hydrophobic compounds such as alkylphenyl ketones. Polymeric phases of very different structure are consistently more retentive toward polar, hydrogen bond donating, and hydrogen bond accepting compounds. The methylene selectivity of the polymeric phases, while it is often lower than SDS micelles in aqueous systems, is maintained to a greater extent in organic-modified buffers. This

is a result of the presence of covalent linkages between the alkyl groups, holding the hydrophobic groups close to each other even in the presence of organic solvents.

Micelle polymers of sodium undecylenate, modified polyallylamine polymers and alkyl modified dendrimers are all observed to undergo a conformational or structural change in organic-modified media. Although the chemical structure of the polymers is covalently stabilized, the conformation of the polymers in solution is determined by a balance between hydrophobic and polar interactions. Adding organic solvents alters the hydrophobic–hydrophilic balance, and permits the polymer to assume a different conformation. This change in conformation affects the migration times, and yields non-linear plots of log k vs. percent organic modifier, but does not dramatically alter the chemical selectivity. It is possible that a structural change does alter the structural selectivity of the SUS polymer, causing different selectivity in acetonitrile vs. methanol modified buffers.

The critical parameters which affect the selectivity of the polymers studied to date include the alkyl chain length and density of alkyl chains on the polymer backbone, the chemistry and density of the ionic groups, the nature of the polymer backbone, and the composition of the buffer medium. The chemistry of the ionic head group made little or no difference in the selectivity of polySUA vs. polySUS or BBMA vs. BMAC, but substitution with L- or D-valine does permit chiral separations. The chemistry and structure of the polymer backbone can have a dramatic effect on selectivity, as was demonstrated with dendritic polymers vs. linear polymers. The nature and concentration of the organic modifier does affect the conformation and selectivity of the polymers. The molecular mass of the phases appears to have minimal effects on the selectivity or affinity of the phases, and thus polydispersity is not considered to be a critical factor in determining the efficiency of the separations.

It has been demonstrated with polymer micelles, acrylate copolymers, dendrimers and polyallylamine phases that polymeric pseudo-stationary phases with unique selectivities can be synthesized using a single polymer backbone. This is an exciting development, which should lead to the design and optimization of a variety of polymeric pseudo-stationary phases in the future.

The technology is directly competitive with capillary electrochromatography [73,74], an approach which has generated a lot of interest recently. One of the advantages of this approach is that polymers with different selectivity can easily be employed, without the need to have a series of packed capillaries with different stationary phases. In contrast, if a selection of polymers were available, employing a pseudo-stationary phase with different selectivity would be as simple as making a solution and rinsing the capillary. Additionally, the use of polymeric pseudo-stationary phases with appropriate selectivity and mobility, combined with optimization of the buffer composition, allows one to fully utilize the advantage of electrokinetic chromatography of having a wide peak spacing at the beginning and a relatively narrow peak spacing at the end of a separation. This characteristic of electrokinetic chromatography is due to the mobility of the pseudo-stationary phase, and permits the separation of a mixture having a wide range of hydrophobic properties in a short time without the need for gradient elution.

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 [50,51], and these structures might be adapted to MEKC to

provide a wide range of chemical selectivities. It has been demonstrated that it is possible to synthesize polymers with altered and unique selectivities. This avenue should be further explored with the synthesis and introduction of polymers with unique selectivity and high electrophoretic mobility. An obvious avenue of work is the synthesis and application of polymers with chiral selectivity. 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 and selectivity. This will facilitate the development and introduction of new polymeric pseudo-stationary phases. Further LSER studies and characterization of the thermodynamics of interaction through Van't Hoff studies would further clarify the nature of the interactions between polymeric phases and analytes. 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 in addition to MEKC.

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